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	Patents ADP number *(if you know it)*	00473587002.
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4	Title of the invention	SODIUM ION CHANNELS
5	Name of your agent *(if you know one)*	MICHAEL A REED (SEE CONTINUATION SHEET)
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11. Michael Reed

I/We request the grant of a patent on the basis of this application

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Sodium ion channels

This invention relates to a novel voltage-gated sodium ion channel, nucleotides coding for it, vectors and host cells containing the same and methods of screening for modulators of said channel for the alleviation of pain and use in hypersensitivity pathologies.

Voltage-gated sodium channels are responsible for the rising phase of the action potential and as such, play a key role in mediating electrical activity in excitable tissues. The sodium channel is activated in response to depolarisation of the membrane. This causes a voltage-dependent conformational change in the channel from a resting, closed conformation to an active conformation, the result of which increases the membrane permeability to sodium ions (1,2).

Voltage-gated sodium channels comprise a multi-subunit complex consisting of a large (230-270kDa) highly glycosylated alpha (α) subunit which is usually associated with one or two of the smaller beta (β) subunits (β1 and β2) (3). The alpha subunits of voltage-gated sodium channels form a large multigene family which has expanded over recent years and at least nine different genes have now been identified in mammals (4-10). This alpha subunit consists of four homologous domains (DI-IV), each containing six potential α-helical transmembrane segments (SI-S6) which make-up the pore forming region. Domains critical for the function of the channel are highly conserved throughout the family of voltage-gated sodium channels. These include the S4 voltage sensors, the loop between domains III and IV which is involved in the inactivation of the channel and the SSI and SS2 segments of the extracellular loop between transmembrane regions S5 and S6, which are responsible for the channels vestibule and ion selectivity (11-13). β subunits appear to have a role in altering the kinetics of the sodium channel during activation and inactivation gating. Expression of the β subunits has been associated with an increase in peak current and a role in trafficking of the a subunit to the membrane (14 –17).

The most potent blocker of voltage gated sodium channels is the puffer fish toxin, tetrodotoxin, (TTX). While most voltage-gated sodium channels are inhibited by low nanomolar concentrations of TTX, there are two channels which are only inhibited by micromolar concentrations of TTX. These are the major cardiac channel (h1 or SKM2) and the sensory neurone specific channel (SNS/PN3) (3,6,7).

Sensory neurones of mammalian dorsal root ganglion (DRG) cells transmit sensory information from the periphery to the central nervous system and are known to express at least three distinct kinetic types of voltage-gated sodium currents (18). The small diameter neurones co-express a rapidly inactivating, fast TTX-sensitive current and a slowly activating and inactivating TTX-resistant sodium current. The larger diameter cells only express TTX-sensitive sodium currents which have intermediate activation and inactivation kinetics (19,20). This electrophysiological analysis has now been supported by molecular distribution studies, which suggest that there is a dynamic expression of voltage-gated sodium channels in DRG neurones which can change during development, response to injury and upon exposure to inflammatory mediators (21-24). The small diameter neurones are unmyelinated and are involved in the transmission of pain impulses, these are the so called c-fibres or nociceptive neurones (25).

Recent experimental evidence has associated and implicated sodium currents with the chronic pain and hypersensitivity pathologies of both inflammatory and neuropathic origin. For example in the small diameter nociceptive neurones, hyperalgesic agents such as prostaglandin E_2 (PGE_2) and serotonin enhance TTX resistant sodium currents and decrease the threshold for inactivation (26-28). Neuronal injury produces dramatic changes in sodium channel expression and distribution, for example accumulation of TTX-sensitive sodium channels at the neuroma of lesioned axons is thought to be responsible for formation of ectopic discharges (29, 30). In each case the neuronal hyperexcitability that results is highly likely to contribute to the induction and maintenance of this sensitised state. It follows that voltage-gated

sodium channels in sensory neurones may provide a highly tractable and attractive target for the development of novel analgesic and anti-hypersensitivity agents.

This supposition is supported by the observation that anaesthetic, anticonvulsant and antiarrythmic drugs, each with sodium channel blocking activity, can produce analgesia. For example, it has been recognised that sub-anaesthetic doses of lignocaine and bupivocaine elevate pain thresholds in man (31,32). In addition the anticonvulsant agents, phenytoin, carbamazepine and the class Ia antiarrythmic agent mexilitene are used clinically for neuropathic pain (33-35). The anticonvulsant lamotrigine is also weakly analgesic (36).

This invention provides a novel voltage-gated sodium channel specifically found in the small diameter subset of mammalian sensory neurones. This novel channel will be termed sensory neurone specific 2a (SNS_{2a}).

Nucleotide sequence analysis of SNS_{2a} reveals a 5298bp open reading frame which encodes a 1765 amino acid protein (Figure 2). This deduced protein sequence shares many of the characteristic features associated with the voltage-gated sodium channel gene family, for example SNS_{2a} contains four homologous repeat domains each comprising six putative membrane spanning segments. A serine residue (S-355) is found at the site critical for TTX sensitivity and based on experiments with SNS/PN3, this residue should confer TTX resistance on clone SNS_{2a} (37). The predicted first intracellular loop region connecting the first and second repeat domains is considerably shorter than the corresponding region in many of the other voltage-gated sodium channels including SNS/PN3, the cardiac channel and the brain channels. Computer generated alignment of SNS_{2a} against the other members of the voltage-gated sodium channel gene family shows this ion channel to be distinct from any of the channels identified to date.

One aspect of the invention therefore provides an isolated mammalian sensory neurone sodium channel protein as set out in Figure 3. Preferably the sodium channel

of the invention is found in the neurones of the dorsal root ganglia. The sodium channel protein may be derived from any mammalian species, preferably the rat or human.

Included within the invention are variants of the sodium channel SNS_{2a}.Such variants include fragments, analogues, derivatives, and splice variants. The term "variant" refers to a protein or part of a protein which retains substantially the same biological function or activity as SNS_{2a}.

Fragments can include a part of SNS_{2a} which retains sufficient identity of the original protein to be effective for example in a screen. Such fragments may be probes such as the ones described hereinafter for the identification of the full length protein. Fragments may be fused to other amino acids or proteins or may be comprised within a larger protein. Such a fragment may be comprised within a precursor protein designed for expression in a host. Therefore in one aspect the term fragment means a portion or portions of a fusion protein or polypeptide derived from SNS_{2a}.

Fragments also include portions of SNS_{2a} characterised by structural or functional attributes of the protein. These may have similar or improved chemical or biological activity or reduced side-effect activity. For example fragments may comprise an alpha helix or alpha -helix forming region, beta sheet and beta-sheet forming region, turn and turn forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, amphipathic regions (alpha or beta), flexible regions, surface-forming regions, substrate binding regions and regions of high antigenic index.

Fragments or portions may be used for producing the corresponding full length protein by peptide synthesis.

Derivatives include naturally occurring allelic variants. An allelic variant is an alternate form of a protein sequence which may have a substitution, deletion or addition of one or more amino acids, which does not substantially alter the function of

the protein. Derivatives can also be non-naturally occurring proteins or fragments in which a number of amino acids have been substituted, deleted or added Proteins or fragments which have at least 70% identity to SNS_{2a} are encompassed within the invention. Preferably the identity is at least 80%, more preferably at least 90% and still more preferably at least or greater than 95% identity for example 97%, 98% or even 99% identity to SNS_{2a}.

Analogues include but are not limited to precursor proteins which can be activated by cleavage of the precursor portion to produce an active mature protein or a fusion with a compound such as polyethylene glycol or a leader/secretory sequence to aid purification.

A splice variant is a protein product of the same gene, generated by alternative splicing of mRNA, that contains additions or deletions within the coding region (Lewin N (1995) Genes V Oxford University Press, Oxford, England).The present invention covers splice variants of the SNS_{2a} sodium channel that occur naturally and which may play a role in changing the activation threshold of the sodium channel.

The protein or variant of the present invention may be a recombinant protein, a natural protein or a synthetic protein, preferably a recombinant protein.

A further aspect of the invention provides an isolated and/or purified nucleotide sequence which encodes a mammalian sodium channel as described above, or a variant thereof. Also included within the invention are anti-sense nucleotides or complementary strands.

Preferably, the nucleotide sequence encodes a rat or human sodium channel. The nucleotide sequence preferably comprises the sequence of the coding portion of the nucleotide sequence shown in Figure 2.

A nucleotide sequence encoding a sodium channel of the present invention may be obtained from a cDNA or a genomic library derived from mammalian sensory neurones, preferably dorsal root ganglia.

The nucleotide sequence may be isolated from a mammalian cell (preferably a human cell), by screening with a probe derived from the rat or human sodium channel sequence, or by other methodologies known in the art such as polymerase chain reaction (PCR) for example on genomic DNA with appropriate oligonucleotide primers derived from or designed based on the rat or human sodium channel sequence and/or relatively conserved regions of known voltage-gated sodium channels. A bacterial artificial chromosome library can be generated using rat or human DNA for the purposes of screening.

The nucleotide sequences of the present invention may be in form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the sodium channel or variant thereof may be identical to the coding sequence set forth in the Figures, or may be a different coding sequence which as a result of the redundancy or degeneracy of the genetic code, encodes the same protein as the sequences set forth therein.

A nucleotide sequence which encodes an SNS_{2a} sodium channel may include: a coding sequence for the full length protein or any variant thereof; a coding sequence for the full length protein or any variant thereof and additional coding sequence such as a leader or secretory sequence or a proprotein sequence; a coding sequence for the full length protein or any variant thereof (and optionally additional coding sequence) and non-coding sequences, such as introns or non-coding sequences 5' and/or 3' of the coding sequence for the full length protein.

The invention also provides nucleotide variants, analogues, derivatives and fragments which encode SNS_{2a}. Nucleotides are included which preferably have at least 70% identity over their entire length to SNS_{2a}. More preferred are those sequences which have at least 80% identity over their entire length to SNS_{2a}. Even more preferred are polynucleotides which demonstrate at least 90% for example 95%, 97%, 98% or 99% identity over their entire length to SNS_{2a}.

The present invention also relates to nucleotide probes constructed from the nucleotide sequences of an SNS_{2a} sodium channel protein or variant thereof. Such probes could be utilised to screen a dorsal root ganglia cDNA or genomic library to isolate a nucleotide sequence encoding an SNS_{2a} sodium channel. The nucleotide probes can include portions of the nucleotide sequence of the SNS_{2a} sodium channel or variant thereof useful for hybridising with mRNA or DNA in assays to detect expression of the SNS_{2a} sodium channel or localise its presence on a chromosome using for example flourescence *in situ* hybridisation (FISH)as described in the examples.

The nucleotide sequences of the invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the protein of the present invention such as hexa-histidine tag or a hemagglutinin (HA) tag or allows determination in screening assays of effective blockage of SNS_{2a} or its modulation.

Nucleotide molecules which hybridise to SNS_{2a}, or to complementary nucleotides thereto also form part of the invention. Hybridisation is preferably under stringent hybridisation conditions. One example of stringent hybridisation conditions which is sometimes used is where attempted hybridisation is carried out at a temperature of from about 35°C to about 65°C using a salt solution which is about 0.9 molar. However, the skilled person will be able to vary such conditions as appropriate in order to take into account variables such as probe length, base composition, type of ions present, etc.

The nucleotide sequences of the present invention may be employed for producing the SNS_{2a} sodium channel protein or variant thereof by recombinant techniques. Thus, for example the nucleotide sequence may be included in any one of a variety of expression vehicles or cloning vehicles, in particular vectors or plasmids for expressing a protein. Such vectors include chromosomal, non-chromosomal and synthetic DNA sequences. Examples of suitable vectors include derivatives of bacterial plasmids; phage DNA: yeast plasmids; vectors derived from combinations of plasmids and phage DNA and viral DNA. However, any other plasmid or vector may be used as long as it is replicable and viable in the host.

More particularly, the present invention also provides recombinant constructs comprising one or more of the nucleotide sequences as described above. The constructs comprise an expression vector, such as a plasmid or viral vector into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises one or more regulatory sequences to direct mRNA synthesis, including, for example, a promoter, operably linked to the sequence. Suitable promoters include: CMV, LTR or SV40 promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector may contain an enhancer and a ribosome binding site for translation initiation and transcription terminator.

Large numbers of suitable vectors and promoters/enhancers, will be known to those of skill in the art, but any plasmid or vector, promoter/enhancer may be used as long as it is replicable and functional in the host.

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts include mammalian expression vectors, insect expression vectors, yeast expression vectors, bacterial expression vectors and viral expression vectors and are described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY., (1989). A preferred vector is pBK-CMV.

The vector may also include appropriate sequences for selection and/or amplification of expression. For this the vector will comprise one or more phenotypic selectable/amplifiable markers. Such markers are also well known to those skilled in the art.

In a further embodiment, the present invention provides host cells capable of expressing a nucleotide sequence of the invention. The host cells can be, for example, a higher eukaryotic cell, such as mammalian cell or a lower eukaryotic cell, such as a yeast cell or a prokaryotic cell such as a bacterial cell. Suitable prokaryotic hosts for transformation include E-coli. Suitable eukaryotic hosts include HEK293 cells.

Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

The SNS_{2a} a sodium channel protein is recovered and purified from recombinant cell cultures by methods known in the art, including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography and lectin chromatography. Protein refolding steps may be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The proteins and nucleotides sequences of the present invention are preferably provided in an isolated form. The term "isolated" means that the material is removed from its original environment (e.g., the naturally-occurring nucleotide sequence or protein present in a living animal is not isolated, but the same nucleotide sequence or protein, separated from some or all of the materials it co-exists with in the natural system, is isolated. Such nucleotide sequence could be part of a vector and/or such nucleotide sequence or protein could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment. The proteins and nucleotides sequences of the present invention are also preferably provided in

purified form, and preferably are purified to at least 50% purity, more preferably about 75% purity, most preferably 90% purity or greater such as 95%, 98% pure.

The present invention also provides antibodies specific for the SNS_{2a} sodium channel. The term antibody as used herein includes all immunoglobulins and fragments thereof which contain recognition sites for antigenic determinants of proteins of the present invention. The antibodies of the present invention may be polyclonal or preferably monoclonal, may be intact antibody molecules or fragments containing the active binding region of the antibody, e.g. Fab or $F(ab)_2$. The present invention also includes chimeric, single chain and humanised antibodies and fusions with non-immunoglobulin molecules. Various procedures known in the art may be used for the production of such antibodies and fragments.

The proteins, their variants especially fragments, derivatives, or analogues thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. Antibodies generated against the SNS_{2a} sodium channel can be obtained by direct injection of the polypeptide into an animal, preferably a non-human. The antibody so obtained will then bind the protein itself. In this manner, even a sequence encoding only a fragment of the protein can then be used to generate antibodies binding the whole native protein. Such antibodies can then be used to locate the protein in tissue expressing that protein.

The antibodies of the present invention may also be of interest in purifying an SNS_{2a} protein and accordingly there is provided a method of purifying an SNS_{2a} or any portion thereof which method comprises the use of an antibody of the present invention.

The present invention also provides methods of identifying modulators of the sodium channel. Screens can be established for SNS_{2a} enabling large numbers of compounds to be studied. High throughput screens may be based on ^{14}C guanidine flux assays and fluorescence based assays as described in more detail below. Secondary screens may

involve electrophysiological assays utilising patch clamp technology or two electrode voltage clamp to identify small molecules, antibodies, peptides, proteins, or other types or compounds that inhibit, block, or otherwise interact with the sodium channel. Tertiary screens may involve the study of the modulators in well characterised rat and mouse models of pain. These models of pain include, but are not restricted to, intra-plantar injection of inflammatory agents such as carageenan, formalin and complete freunds adjuvant (CFA). Models of neuropathic pain such as loose ligature of the sciatic nerve are also included.

The invention therefore provides a method of assaying for a modulator comprising contacting a test compound with the sodium channel and detecting the activity or inactivity of the sodium channel. Preferably, the methods of identifying modulators or screening assays employ transformed host cells that express the sodium channel. Typically, such assays will detect changes in the activity of the sodium channel due to the test compound, thus identifying modulators of the sodium channel.

For example, host cells expressing the sodium channel can be employed in ion flux assays such as $^{22}Na+$ ion flux and ^{14}C guandinium ion assays, as described in the examples and in the art, as well as the SFBI fluorescent sodium incubator assays as described in Levi et al., (1994) J Cardiovascular Electrophysiology 5-241-257 and voltage sensing dyes such as DiBAC. Host cells expressing the SNS_{2a} sodium channel can also be employed in binding assays such as the 3-H- batrachotoxin binding assay described in Sheldon et al., (1986) Molecular Pharmacology 30:617-623; the 3-H- saxitoxin assay as described in Rogart et al (1983) Proc Natl, Acad, Sci, USA 80: 1106-1110: and the scorpion toxin assay described in West et al., (1992) Neuron 8: 59-70.

In general, a test compound is added to the assay and its effect on sodium flux is determined or the test compound's ability to competitively bind to the sodium channel is assessed. Test compounds having the desired effect on the sodium channel are then selected.

Modulators of the sodium channel will prevent the transmission of impulses along sensory neurones and thereby be useful in the treatment of acute, chronic or neuropathic pain and or in the treatment of hypersensitivity pathologies. The invention therefore provides a modulator of a protein or a variant thereof as described above identifiable by a method described above for use in therapy. The invention further provides the use of a modulator of a sodium channel protein optionally identifiable by a method described above for the manufacture of an analgesic or anti-hypersensitvity medicament. Moreover the invention provides a method of treatment which comprises administering to a patient an effective amount of a modulator of a protein as described above.

Complementary or anti-sense strands of the nucleotide sequences as hereinabove defined can be used in gene therapy. For example, the cDNA sequence or fragments thereof could be used in gene therapy strategies to down regulate the sodium channel. Antisense technology can be used to control gene expression through triple-helix formation of antisense DNA or RNA, both of which methods are based on binding of a nucleotide sequence to DNA or RNA.

A DNA oligonucleotide is designed to be complimentary to a region of the gene involved in transcription thereby preventing transcription and the product of the sodium channel. The antisense RNA olignucleotide hybridises to the mRNA in vivo and blocks translation of the mRNA into the sodium channel. Antisense oligonucleotides or an antisense construct driven by a strong constitutive promoter expressed in the target sensory neurons would be delivered either peripherally or to the spinal cord.

The regulatory regions controlling expression of the sodium channel gene could be used in gene therapy to control expression of a therapeutic construct in cells expressing the sodium channel.

Figures

Brief description of the Figures:

Figure 1 is a summary of the rat SNS_{2a} ion channel fragments isolated, and probes used for analysis

Figure 2 shows the complete DNA nucleotide sequence including the 5298 bp open reading frame (base 49 - 5347) of the rat SNS_{2a} ion channel nucleotide sequence.

Figure 3 shows the nucleotide and encoded amino acid sequence of the rat SNS_{2a} ion channel protein.

Figure 4 shows the amino acid sequence of rat SNS_{2a}; the shading denotes predicted transmembrane regions; the critical serine (S-355) site involved in tetrodotoxin (TTX) sensitivity is in bold and the potential cAMP dependent protein kinase phosphorylation sites are marked with an emboldened diamond.

Figure 5 shows multiple sequence alignment of SNS_{2a} against the voltage-gated sodium channel gene family. The shaded regions denote predicted transmembrane regions. The genes are as described in References 4-7 and are as follows: rbi = rat brain 1 sodium channel; : rbii = rat brain 2 sodium channel; : rbiii = rat brain 3 sodium channel; pn1= Peripheral neuronal 1 sodium channel; nach6 = sodium channel 6; skm1 = skeletal muscle 1 sodium channel; pn3 = Peripheral neuronal 3 sodium channel; Cardiac = Cardiac sodium channel; SNS_{2a} = Sensory sodium channel 2a; Glial = Glial sodium channel.

Figure 6 shows a dendrogram of relative homology between the ion channels generated from the multiple sequence alignment in Figure 5.

Figures 7a -7g shows the position of the human SNS_{2a} sequences lined up against the rat cDNA clones.

Figure 8 shows the localisation of human SNS_{2a} to human chromosome 3p21.

Figure 9 shows rat multiple tissue Northern Blot probed with SNS_{2a}. Lane 1 = DRG; Lane 2 = Spinal cord; Lane 3 = Total brain; Lane 4 = Adrenal gland; Lane 5 = Heart; Lane 6 = PC12; Lane 7 = PC12 + NGF; Lane 8 = RNA markers.

Figure 10 In situ hybridisation in rat DRG tissue using an SNS_{2a} specific probe. Figure 10a) shows a sense probe and b) shows an anti-sense probe.

Figure 11 shows localisation of SNS_{2a} to human DRG

Figure 12 Northern blot probed with SNS_{2a} using DRG tissue taken from rat pain models. Lane 1 = Control DRG; Lane 2 = DRG + 24 hours complete freunds adjuvant (CFA); Lane 3 = DRG + 24 hours sciatic nerve cut; Lane 4 = DRG + 48 hours sciatic nerve cut; Lane 5 = DRG + 7 days sciatic nerve cut.

The following examples are for illustrative purposes only and are not limiting of the invention.

Example 1: DRG cDNA Library screening

Example 1a: Obtaining The Probe

A sodium channel probe was generated to allow screening of a rat DRG cDNA library with the aim to identify novel sodium channels present in the DRG. A pan specific sodium channel probe was obtained from Polymerase chain reaction (PCR) experiments using rat genomic DNA as the template and degenerate PCR primers designed from within the 3' coding regions of the brain II, heart, skeletal muscle and glial voltage-gated sodium channel. The oligonucleotide primers used for this

analysis were as follows, FORWARD PRIMER (5' CCTG/CGTCATGTTCATCTAC 3', and REVERSE PRIMER (5' CTCATAA/GGAA/GAC/TCTTGGAG/AGGG 3'). The PCR conditons used, were 94°C for 30 seconds, 50°C for 1 minute and 72°C for 2 minutes. These conditions were used for 35 cycles of PCR. The resulting PCR products were separated on a 1% agarose gel and cloned into the TA cloning kit (Invitrogen) according to manufacturers instructions. The resulting clones were taken for sequence analysis and separate clones were identified with identical sequence to the published rat brain II, heart, skeletal muscle and glial voltage-gated sodium channels.

A rat DRG cDNA library was constructed in λZAP Express™ Bacteriophage system (Stratagene), allowing it to be directionally cloned within the pBK-CMV excision vector. Briefly, lumbar DRG tissue was removed from adult rats and frozen in liquid nitrogen until ready for processing. Total RNA was extracted using RNAzol B (Biogenesis) according to the manufacturers instructions. This method is based on the guanidine isothiocyanante and phenol/chloroform extraction method developed by Chomczynski and Sacchi, Analytical Biochemistry (1987) **162**, 156-169. Poly (A+) RNA was then isolated from the total RNA pool by oligo dT celluloise chromatography. (invitrogen) as per manufacturers instructions. 5μg of this poly (A+) rat DRG RNA was used as the starting template for cDNA library synthesis. This was carried out exactly as stated in the Stratagene Instruction manual for construction of a ZAP express cDNA library using the Gigapack III Gold cloning kit.

Initially two million plaque forming units from this library were screened (as outlined in DNA transfer and hybridisation and probing) with the pan specific sodium channel probe. The resulting positive plaques were purified to homogeneity (as outlined in the Stratagene instruction manual for the construction of a ZAP express cDNA library using the Gigapack III Gold cloning kit) and subjected to sequence analysis. Several clones were obtained which demonstrated a novel sequence related to voltage-gated sodium channels. The longest of these clones has been annotated as LARI/QFL in figure 1. Figure 1 displays the key clones obtained from the DRG cDNA library

screening. This novel sequence was a fragment of the sodium channel referred to in this invention as SNS_{2a}

Subsequently, a further one and a half million plaques were screened using the probe (LARI/QFL), specific to this novel sodium channel. Further positive clones were obtained and verified by sequence analysis. The largest of these clones designated as clone 63.1 in figure 1 was 3.6 kb in length. Degenerate oligonucleotide primers were designed to perform RT-PCR reactions on DRG RNA. The primers used were as follows 5' AGGGAGGTCACCGGCCTGAAA/C 3' and 5' AGTGGATA/CGAGAA/CCATGTGGG 3'. Conditions used were 94° C for 30 seconds, 50°C for 1 minute and 72°C for 2 minutes. These conditions were used for 35 cycles of PCR. The resulting PCR products were separated on a 1% agarose gel and cloned into the TA cloning kit (Invitrogen) according to manufacturers instructions. The resulting clones were taken for sequence analysis. This resulted in the discovery of the partial SNS_{2a} clone 18/14. This is annotated as 18/14 in figure 1 which illustrates the position of this clone relative to the full length sequence of SNS_{2a} Two million plaques were screened in the third cDNA library screening using this probe designated as 18/14, (probe labelling as in hybridisation and probing). Analysis of the positive clones obtained from this screen resulted in the discovery of the fragments annotated in figure 1 as 16/24, 31/42 and the 3.4kb clone 71/72. The two clones designated 71/72 and 63.1 (figure 1) overlapped with each other thus allowing them to be joined together using a unique Bgl II (New England Biolabs) restriction site found from position 2895 bp to 2900 bp of SNS_{2a}. This step generated the full length SNS_{2a} clone which is shown in figure 2.

SNS_{2a} has been assembled in the EcoRI/XhoI sites of the mammalian expression vector pBK-CMV (Stratagene). This allows for both transient and stable expression studies in mammalian cells such as HEK293 cells (ATCC).

Nucleotide sequence analysis of SNS_{2a} reveals a 5298bp open reading frame which encodes a 1765 amino acid protein (figures 2 and 3). This deduced protein sequence

shares many of the characteristic features associated with the voltage-gated sodium channel gene family, however, the predicted first intracellular loop region connecting the first and second repeat domains is considerably shorter than the corresponding region in many of the other voltage-gated sodium channels including SNS/PN3, the cardiac channel and the brain channels (figure 4). Figure 5 shows a computer generated alignment of SNS_{2a} against the other members of the voltage-gated sodium channel gene family. Figure 6 shows the dendrogram generated from this alignment and depicts the relative similarity of the channels to each other.

Example 1b: DNA Transfer

The DNA was transferred onto a GeneScreen™ hybridisation transfer membrane (DUPONT) by placing on the surface of the phage infected plate for 1 minute. The membrane is washed with 1M NaOH twice for 2 minutes, followed by two neutralisation steps in 1M Tris (pH 7.4) for an additional 2 minutes. An additional duplicate lift was done with the filter on the plate for five minutes prior to the washing steps. The membrane is then air dried overnight or crosslinked using the UV Stratalinker (Stratagene).

Example 1c: Hybridisation and probing

The membranes were hybridised for 4 hours shaking at 60°C in a 10% dextran sulphate, 1% lauryl sulphate (SDS)(see solutions and media) and 1M NaCl solution. The probes used were LARI & QFL and 18/14 respectively, from the 5' and middle regions of 33b. The probe was labelled with [α ^{32P}] dCTP (Amersham) using the Rediprime™ DNA labelling system (Amersham), so as to obtain approximately 500,000 cpm of the labelled probe per ml of prehybridization solution. Briefly, 100ng of each probe was boiled for 3 minutes (denaturization) and then cooled on ice for 2 minutes in a total volume of 45µl. This was added to the labeling tube from the kit together with 3µl of 32P dCTP, followed by an incubation at 37 °C for 30 minutes. 400µl of Herring Sperm DNA (Sigma) at a concentration of 400µg for 50ml was added to the labelled probe and heated at 99 °C for 3 minutes followed by rapid

cooling on ice. The labelled probe was added and mixed well in the prehybridisation solution. The membranes were hybridised overnight at 55 °C.

The membranes were then washed, first at room temperature, in 2x SSC (3M sodium chloride and 0.3M sodium citrate pH7) and 1% SDS (sodium dodecyl sulphate) for 5 minutes, followed by 2x SSC and 1%SDS for 30 mins at 50 °C, and if necessary further washes with 1x SSC and 0.5% SDS or 0.1x SSC and 0.1% SDS for 30 mins at the same temperature. The membranes were then exposed to Scientific Imaging Film-AR (Kodak) using intensifying screens at -70 °C overnight and the film developed.

Example 1d: Southern Blot analysis

PCR products which were separated using agarose gel electrophoresis were denatured in situ by shaking the gel slowly in 1.5M NaCl for 10 minutes followed by a 0.5M NaOH solution for 30 minutes. DNA transfer onto a GeneScreen™ hybridization transfer membrane (DUPONT) by capillary action occurred overnight, followed by washing in 2x SSC for 2 minutes and left to air dry. The hybridization and probing was carried out in the same way as for the library screening.

Example 2: *Iv vivo* excision analysis

Approximately 6 phage plugs were removed from the agarose plate and placed in 500µl of SM buffer. Elution of the phage particles occurred at room temperature while gently shaking for 2-3 hours. 1µl of ExAssist™ Helper phage (Stratagene) was added to 100µl of phage stock in SM buffer (see media and solutions) and incubated at 37 °C for 15 minutes. 3ml of liquid broth (see media and solutions) was added, followed by shaking at 225rpm at 37 °C for 3 hours. Heat shock at 70 °C for 15 minutes was followed by centrifugation at 4000rpm for 15 minutes at 4°C. The supernatant was carefully decanted into a sterile 50ml falcon tube and stored at 4°C until needed.

10µl and 100µl of the rescued recombinant plasmid (supernatant from the step above) was used to transform 200µl of XLOLR cells (Stratagene) at OD_{600} 1.0 and incubated

at 37 °C for 15 minutes. The samples are incubated for a further 45 minutes at 37 °C after the addition of 300μl of L-broth (see media and solutions), followed by spreading on kan-plates (15μg/ml) (see media and solutions) and incubation overnight at 37°C. Positive colonies were analysed by digest analysis using XhoI and EcoRI restriction enzymes followed by subsequent southern blot analysis.

Example 3: Transient expression of SNS_{2a} in mammalian cells

Mammalian cells such as HEK293 cells should be plated 24 hours prior to transfection, such that they are 50 – 80% confluent for the transfection procedure. On the day of transfection fresh media should be added to the cells. The transfection protocol to be used will rely upon the calcium phosphate transfection method (CalPhos maximer, Clontech) although any transient transfection method can be used. Briefly, a solution referred to as solution A, will be made up containing 2- 4 μg of plasmid DNA per 4 x 10^5 cells, 5 – 30 μl of CalPhos maximer, 12.4 μl 2M calcium solution, sterile water to 100μl. The following solution referred to as solution B will also be made up comprising, 100 μl of HEPES buffered saline. Solution B will then be carefully vortexed while solution A will be added dropwise. The mixed solutions will be incubated at room temperature for 20 minutes. After this period the solution will be gently vortexed and added to the cell culture medium. 200 μl of solution will be used per 35 mm^2 vessel with 4 x 10^5 cells. The vessel can then be gently rocked to distribute the solution. The cells will be incubated at 37^0 C for 2 – 6 hours, after which the medium wll be removed by aspiration and the cells will be washed with phosphate buffered saline. Fresh culture media will then be added to the cells.
Electrophysiological assays can then be carried out 24 – 72 hours post transfection or alternatively antibiotic selection can be applied after 24 hours if stable cell lines are required .

Example 4: Northern blot analysis

20μg of total RNA from DRG, heart, spinal cord, adrenal glands, PC12 cells (ATCC), and PC12 cells pretreated with NGF were electrophoresed on a 1% agarose gel, containing 8% formaldehyde. (The preparation of the total RNA was carried out as

described in the construction of the rat DRG cDNA library) The gel was then blotted onto a Genescreen™ membrane as described previously in Example 1d and probed with the 18/14 probe as described in Example 1c. Exposure to Kodak X-AR film occurred overnight.

The results of this Northern blot analysis using the 18/14 probe, which was specific to SNS_{2a} demonstrated a transcript size of approximately 9kb in DRG cells, while no expression was observed in spinal cord, brain, adrenal gland, heart and the rat pheochromocytoma cell line (PC12) in the absence or presence of nerve growth factor (NGF) (figure 9). *In situ* hybridisation experiments performed on DRG sections demonstrated that SNS_{2a} expression was limited to the small diameter cells (figure 10). Similar *in situ* hybridisation experiments were performed on spinal cord and whole brain sections and no specific labelling was observed confirming the Northern analysis work.

The expression of SNS_{2a} in DRG tissue was studied in DRG tissue removed from two separate rat models of pain, namely the Complete Freunds Adjuvant (CFA) model and the sciatic nerve cut (axotomy) model. The expression of SNS_{2a} was studied by Northern blot analysis using the probe 18/14 as described earlier in this section. In the CFA model at the 24 hour time point, there was a significant increase in expression of SNS_{2a} however there was a significant decrease in the level of SNS_{2a} mRNA at the 48 hour and 7 day time periods in the axotomy model (figure 12). This important series of experiments demonstrates differential regulation of this novel channel SNS_{2a} in well characterised models of pain.

Example 5: Antibody Generation

The octadecapeptide CNGDLSSLDVAKVKVHND relating to amino acid residues 1748 to 1765 of SNS_{2a} and the peptide EERYYPVIFPDERNC relating to amino acid residues 2 to 15 of SNS_{2a} were synthesised on a Biosearch 9500 peptide synthesiser using solid-phase Fmoc chemistry under conditions recommended by the suppliers. Cleaved peptide was purified by gel filtration and conjugated to purified

protein derivative of tuberculin (PPD) using sulpho-SMCC. Dutch rabbits, presensitised against BCG, were immunised with the resulting conjugate emulsified in incomplete Freunds adjuvant. Rabbits were boosted at three week intervals and serum prepared from test bleeds 7 days after each injection. The specific antibody response was followed by indirect ELISA using free synthetic peptide as antigen. High titre antisera were used for further studies.

These anti-peptide antibodies directed to SNS_{2a} can been used in immunohistochemistry experiments. Several fusion protein antibodies have also been generated against SNS_{2a} The PCR primers used to generate fusion peptides were as follows:

Fusion peptide 1 5' GATCGAATTCAAGGAGAAAATGTTTCAGGA 3'and
5' GATCGTCGACTCATTTGGTCTGCTCAAGGA 3'
Fusion peptide 2 5' GATCGAATTCGGCGGTGCCCTACCCACCTC 3' and
5' GATCGTCGACTCATTCCATTTCAACCCCTT 3'
Fusion peptide 3 5' GATCGAATTCAAGCACAACTGTGGCCCCAA 3' and
5' GATCGTCGACTCACATTATGAAGTCTTCGC 3'

The anti-peptide antibodies have been verified by specific staining to recombinant SNS_{2a} expressed in HEK293 cells (see section on transient expression of SNS_{2a}). The anti-peptide antibodies have also been used to stain rat DRG sections and acutely dissociated rat DRG cells. Once again the antibody recognises the small diameter cell bodies of the peripheral sensory neurones. This observation has been extended to human DRG tissue and this experiment demonstrates that the antibodies raised to the rat sequence do in fact cross react with the human SNS_{2a} channel (figure 11).

Example 6: Electrophysiology

Following successful transfection of mammalian cell with SNS_{2a} the following electrophysiological experiments can be carried out.

All experiments will be performed at room temperature (20-22°C).. Drugs will be applied either via addition to the bath perfusate or using a rapid perfusion system

which will consist of a series of reservoirs connected to a small microfil tube. Whole-cell currents will be recorded using an Axopatch 200B amplifier (Axon Instruments; Hamill *et al.,* 1981). Patch pipettes will be fabricated from 1.5mm outside diameter borosilicate capillary glass (Clark Electromedical) using a micropipette puller (Sutter model P97), and fire polished (Narishige Microforge) to give final tip resistances of 2-4mΩ. A silver/silver chloride pellet will be used as the bath reference electrode and the potential difference between this and the recording electrode will be adjusted for zero current flow before seal formation. Cells can be visualised using a Diaphot200 inverted microscope (Nikon) with modulation contrast optics at a final magnification of x400. High resistance seals (1-10GΩ) between pipette and neuronal cell membranes are achieved by gentle suction, and the 'whole cell' configuration attained by applying further suction.

Voltage command protocols will be generated, and current records stored, via a digidata1200 analog/digital interface (Axon Instruments) controlled by microcomputer (Viglen Pentium) using pCLAMP6 Clampex software (Axon Instruments). Signals will be prefiltered at 5kHz bandwidth and sampled at 20kHz. Capacitance transients and series resistance errors are compensated for (80-85%) using the amplifier circuitry, and linear leakage currents will be subtracted using an on-line 'P-4' procedure provided by the commercial software package. In most cases evoked Na^+ currents should range from -600pA to -4500pA and thus the maximum estimated voltage drop across the compensated series resistance will amount to less than 4mV.

Example 6b: Analysis of data

Data will be analysed using pCLAMP6 (Clampfit), ORIGIN and DAISI data handling and graphical presentation software packages. Results will be presented as either arithmetic mean ± s.e. mean or geometric mean with 95% confidence limits.

Statistical comparisons will be made using paired or unpaired Student's t-test and considered of significance when $P<0.05$.

For construction of activation curves, Na^+ conductance (g_{Na}) will be calculated from the peak current (I_{Na}), according to the following equation: $g_{Na} = I_{Na}/V-E_{Na}$ where V is the test pulse potential and E_{Na} the membrane potential at which the peak current is reversed. Normalised Na^+ conductance can be plotted against test pulse potentials and fitted to a Boltzman function according to the equation; $g/g_{Max} = 1/[1+\exp(V_{1/2}-V/k)$ where g is the measured conductance, g_{Max} is the maximal conductance, $V_{1/2}$ is the membrane potential at which the half-maximal channel open probability occurs and k is the slope of the curve. For construction of inactivation curves, the peak current (I) will be normalised relative to the maximal value (I_{Max}) obtained at a holding potential (V_h) of -90mV and plotted against the conditioning pulse potential. Data will be fitted by a Boltzman function according to the following equation: $I/I_{Max} = 1/[1+\exp(V_{1/2}-V/k)$ where V is the membrane potential during prepulses, $V_{1/2}$ the potential at which the half-maximal channel inactivation occurs and k the slope of the line.

For fitting drug concentration-response curves, an independent binding site model of the form; $I = a-d/1+(x/IC_{50})^b) + d$ will be used, where I is the current in the presence of drug, a the normalised peak current before drug, b the Hill slope value, d the maximum inhibitory effect, x the drug concentration and IC_{50} the drug concentration required to produce 50% current inhibition. To assess current kinetics, time constant (τ) values defined as the time to achieve 50% current activation (τ_{act}) and 50% inactivation (τ_{inact}) will be obtained by fitting the respective phases of the current traces to single exponential functions of the order; $A \times \exp[-t/\tau] + C$ where A is the current amplitude at the start of the fitting region, t the time and C the steady-state asymptote. Best fits will be obtained using the Chebyshev transformation non-iterative curve fitting technique.

The following drugs and solutions will be used in such a study; sodium chloride (NaCl), potassium chloride (KCl), choline chloride, magnesium chloride heptahydrate

($MgCl_2$), N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), HEPES-Na, laminin, tetrodotoxin, poly-DL-ornithine hydrobromide (all Sigma), ethylene glcycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA; Fluka Biochemika), calcium chloride ($CaCl_2$; BDH Chemicals), tetraethylammonium chloride (TEA), caesium fluoride (CsF; Aldrich Chemical Co.), collagenase type III (130units mg^{-1}), trypsin TPCK (226units mg^{-1}; Worthington Biochemical corporation), All drugs and chemicals will be dissolved in distilled water (or cell culture media where appropriate).

Example 7: Screening

Having established that SNS_{2a} has significant potential as a pain target a screening strategy has been determined in order to identify modulators of channel function. High throughput screens are based on assays such as ^{14}C guanidine flux assays and fluorescence based assays using both sodium indicator dyes such as SBFI and voltage sensing dyes such as DiBAC. Secondary screens involve electrophysiological assays utilising patch clamp technology or two electrode voltage clamp. Tertiary screens involve the study of modulators in rat and mouse models of pain.

The critical path depicting the key steps in the SNS_{2a} high throughput screen is shown below. The screen should aim to cover at least 200,000 compounds in the primary screen but may be as high as 1 million compounds, the hit compounds are then re-tested against mammalian cell lines expressing the brain and/or cardiac sodium channels. The tertiary screen will take compounds which are potent and selective and test them in a range of in-vivo pain models.

		Primary Screen		Secondary Screen		Tertiary Screen
Compounds	>200K →	**HTS:- FLIPR/ G-Flux**	→	**Selectivity/ Use Dependence**	→	**In-vivo**
		Recombinant SNS_{2a} Cell Line		*Brain/Cardiac (SKN-SH-SY5Y) N-Type Calcium*		*Isolated Nerve Inflammatory Pain Neuropathic Pain HT Patch Further Selectivity Detailed Electrophysiology*

The G-FLUX methodis the method of choice and it has been further improved with the introduction of Cytostar-T plates (Amersham) which remove the necessity for digestion of the cells in triton and transfer into scintillation vials. Cytostar-T plates are standard format tissue culture treated plates in which the transparent base of each well is composed of polystyrene and scintillant that permits cultivation and observation of adherent cell monolayers. Radioisotopes brought in close proximity with the base by virtue of the biological process within the cells thereby result in the generation of light.

Guanidine Flux (G-Flux) assay

Mammalian cells stably over-expressing SNS_{2a} will be cultured in 96 well plates. One T225cm ^{3} flask will be sufficient for setting up ten 96 well plates with a volume of 100μl cell culture medium in each well.These plates are set up the night before each assay run. The culture medium is removed and 100μl of assay buffer (125mM Choline chloride, 50mM HEPES, 5.5mM Glucose, 0.8mM $MgSO_4$, 5mM KCl, pH 7.4) added. The test compounds are then added to the wells and pre-incubated for a period of 10 minutes. Scorpion toxin (0.31 mg ml^{-1}) and veratrine (1.25mg ml^{-1}) (Sigma) will then be added to activate the sodium channel, these compounds hold the channel in an open conformation. The cells are incubated for a further 10 minutes

prior to the addition of ^{14}C guanidine (Amersham). This is incubated for a period of 3 minutes after which time the whole plate can be read on a scintilation counter.

Example 8: Cloning of human SNS_{2a}

The human SNS_{2a} gene has been cloned as a genomic DNA fragment. PCR experiments were performed on human genomic DNA, using oligonucleotide primers designed form the rat SNS_{2a} sequence. A fragment corresponding to the human SNS_{2a} gene was subsequently isolated and sequenced. A human bacterial artificial chromosome library (Research Genetics) was then screened using PCR primers designed from the human sequence. A 120kb BAC clone (BAC#4) was isolated which has been extensively characterised following the construction of a random library from the BAC clone. (see section below) This clone contains the gene encoding human SNS_{2a}, figure 7a shows regions where coding sequence has been obtained from the BAC clone against an idealised template. Figure 7b-g shows the actual DNA sequences obtained for human SNS2a lined up against the rat SNS_{2a} template.

This BAC clone (BAC#4) containing human SNS_{2a} was mapped to human chromosome 3p21 by fluorescence in situ hybridisation (FISH) (figure 8). The human SNS/PN3 gene has also been mapped to the same chromosomal locus. It is worthy of note that the human cardiac channel has also been mapped to chromosome 3p21. A new gene cluster of TTX- resistant sodium channels has therefore been identified on human chromosome 3.

Example 9: Purification of BAC DNA

BAC DNA was purified according to the Qiagen BAC DNA method. Briefly BAC liquid culture was inoculated into a 5ml starter culture of L broth with12.5 μg/ml chloramphenicol selection. This was used to inoculate 200ml L broth with (selection) which was then grown for 14 hours at 37 ^{0} C with vigorous shaking. The culture was then centrifuged at 4500 x g fo 20 minutes. The bacterial pellet was resuspended in 20 ml of buffer P1. 20 ml of P2 was added and the solution was mixed gently and

incubated at 21 ^{0} C for 5 minutes 20 ml of chilled buffer P3 was added, solution mixed gently and incubated on ice for 15 minutes. Following centrifugation at 20000 x g for 30 minutes the supernatant was applied to an equilibrated Qiagen Tip 100. The column was washed with twice with 10 ml of buffer QC The DNA was eluted with five 1 ml aliquots of buffer QF, pre warmed to 65 ^{0} C . The DNA was precipitated with 3.5 ml of isopropanol and centrifuged at 15000 x g for 15 minutes. The supernatant was removed and the pellet was washed with 2 ml of 70 % ethanol and centrifuged at 1500 x g for 10 minutes. The pellet was finally air dried for 10 minutes and resuspended in water.

Example 10: Construction of Random Library from BAC Clone

This was an essential prerequisite to analyse the 120kb BAC clone containing the human SNS_{2a} gene.

5μg of BAC DNA in a volume of 50μl was sonnicated in the cup horn, in two pulses of 1 second at power level 2, with cooling on ice for 1 minute between pulses. The overhanging or ragged ends, caused by the sonication, of the fragmented DNA molecules were made flush by the exonuclease or polymerase activity of T4 DNA polymerase. The components were as follows ,47.5 μl sonicated DNA, 20 μl 5 x T4 DNA buffer, 10 ul 2 mM each dNTP , 17.5 μl double distilled water, 5 ul T4 DNA pol (1unit/μl Boehringer) This reaction mix was incubated at 37 ^{0} C for 3 hours. The DNA was size selected with a Pharmacia SizeSep 400 spin column. The resulting DNA fragments were ligated into a SmaI phosphatased pBluescript II SK vector (Stratagene) and subsequently transformed into XL1 blue competent E.coli (Stratagene). Individual colonies are PCR amplified with M13 reverse and M13 –20 primers,which flank the insert. The PCR products were sequenced using the nested primers T3 and T7.

A second method was employed as above except that following T4 DNA polymerase repair, oligonucleotide linkers were ligated onto the DNA fragments. Using primers directed against sites within these oligos the DNA fragments were amplified by PCR. The lnker ligation reaction mix was set up as follows, l of sonicated BAC DNA, 5μl

T4 DNA ligase (400 units/μl NEB), 5 μl 10 x ligase buffer , 2μl linkers, 37.5 μl double distilled water , and icubated for 8 hours at 21 ^{0} C. PCR amplification was performed using 50 p.moles linker primers, 1 x buffer (Promega), 1.5 mM $MgCl_2$, 200 μM each dNTP, Taq (Promega) 0.5 unit. The reaction volume was 50 μl and the PCR parameters: 94 ^{0} C 2 minutes, 94 ^{0} C 30 seconds, 55 ^{0} C 1 minute, 72 ^{0} C 2 minutes, for 40 cycles, 72 ^{0} C 10 minutes. The resulting PCR products were ligated into the TA cloning vector (Invitrogen) and transformed in INVαF' competent E.coli (Invitrogen). The resulting PCR products were then sequenced with T3 and T7, which are nested primers.

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Claims

1. An isolated mammalian sodium ion channel protein comprising the amino acid sequence shown in Figure 3 or a variant thereof.

2. A sodium channel protein or variant thereof according to claim 1 which is a rat protein.

3. A sodium channel protein or variant thereof according to claim 1 which is a human protein.

4. A sodium channel protein or variant thereof according to any of claims 1-3 for use in a method of screening for agents with analgesic or anti-hypersensitivity activity.

5. A nucleotide sequence encoding a sodium channel protein or a variant thereof according to any of the preceding claims, or a complementary strand thereto.

6. A nucleotide sequence according to claim 4 wherein the sequence is as shown in Figure 2 or is a variant thereof.

7. A nucleotide sequence that hybridises to any part of a nucleotide strand referred to in either of claims 5 or 6.

8. A vector comprising a nucleotide sequence according to any of claims 5-7.

9. A host cell transfected with a vector according to claim 8.

10. An antibody specific for a sodium channel protein or variant thereof according to any of claims 1- 3.

11.A method for the identification of a modulator of a sodium channel according to any of claims 1- 3 or a variant thereof comprising contacting said channel with a test compound and detecting activity or inactivity of said channel.

12.A method of assaying compounds which modulate sodium flux comprising expressing a protein or variant thereof according to any of claims 1-3 in a host cell; contacting said protein with a potential modulator; and measuring sodium flux.

13.A modulator of a protein or a variant thereof as defined in any of claims 1-3 identifiable by a method according to any of claims for use in therapy.

14.Use of a modulator of a protein as defined in any of claims 1-3 identifiable by a method according to any of claims for the manufacture of an analgesic or anti-hypersensitvity medicament.

15.A method of treatment which comprises administering to a patient an effective amount of a modulator of a protein as defined in any of claims 1-3 identifiable by a any of the methods according to claims.

Abstract

This invention relates to a novel voltage-gated sodium ion channel specifically found in the small diameter subset of mammalian sensory neurones termed sensory neurone specific 2a (SNS_{2a}). Nucleotides coding for it, vectors and host cells containing the same are also claimed, including methods of screening said channel to identify modulators which can be used in the alleviation of pain and/or in the treatment of hypersensitivity pathologies.

Figure 2

1	GGAGCCATAC	GGTGCCCTGA	TCCTCTGTAC	CAGGAAGACA	GGGTGAAGAT
51	GGAGGAGAGG	TACTACCCGG	TGATCTTCCC	GGACGAGCGG	AATTTCCGCC
101	CCTTCACTTC	CGACTCTCTG	GCTGCCATAA	AGAAGCGGAT	TGCTATCCAA
151	AAGGAGAGGA	AGAAGTCCAA	AGACAAGGCG	GCAGCTGAGC	CCCAGCCTCG
201	GCCTCAGCTT	GACCTAAAGG	CCTCCAGGAA	GTTACCTAAG	CTTTATGGTG
251	ACATTCCCCC	TGAGCTTGTT	ACGAAACCTC	TGGAGGACCT	GGACCCCTAC
301	TACAAAGACC	ATAAGACATT	CATGGTGTTG	AACAAGAAAA	GAACAATTTA
351	TCGCTTCAGC	GCCAAGCGGG	CCTTGTTCAT	TCTGGGGCCT	TTTAATCCCC
401	TCAGAAGCTT	AATGATTCGT	ATCTCTGTCC	ATTCAGTCTT	TAGCATGTTC
451	ATCATCTGCA	CGGTGATCAT	CAACTGTATG	TTCATGGCGA	ATTCTATGGA
501	GAGAAGTTTC	GACAACGACA	TTCCCGAATA	CGTCTTCATT	GGGATTTATA
551	TTTTAGAAGC	TGTGATTAAA	ATATTGGCAA	GAGGCTTCAT	TGTGGATGAG
601	TTTTCCTTCC	TCCGAGATCC	GTGGAACTGG	CTGGACTTCA	TTGTCATTGG
651	AACAGCGATC	GCAACTTGTT	TTCCGGGCAG	CCAAGTCAAT	CTTTCAGCTC
701	TTCGTACCTT	CCGAGTGTTC	AGAGCTCTGA	AGGCGATTTC	AGTTATCTCA
751	GGTCTGAAGG	TCATCGTAGG	TGCCCTGCTG	CGCTCGGTGA	AGAAGCTGGT
801	AGACGTGATG	GTCCTCACTC	TCTTCTGCCT	CAGCATCTTT	GCCCTGGTCG
851	GTCAGCAGCT	GTTCATGGGA	ATTCTGAACC	AGAAGTGTAT	TAAGCACAAC
901	TGTGGCCCCA	ACCCTGCATC	CAACAAGGAT	TGCTTTGAAA	AGGAAAAAGA
951	TAGCGAAGAC	TTCATAATGT	GTGGTACCTG	GCTCGGCAGC	AGACCCTGTC
1001	CCAATGGTTC	TACGTGCGAT	AAAACCACAT	TGAACCCAGA	CAATAATTAT
1051	ACAAAGTTTG	ACAACTTTGG	CTGGTCCTTT	CTCGCCATGT	TCCGGGTTAT
1101	GACTCAAGAC	TCCTGGGAGA	GGCTTTACCG	ACAGATCCTG	CGGACCTCTG
1151	GGATCTACTT	TGTCTTCTTC	TTCGTGGTGG	TCATCTTCCT	GGGCTCCTTC
1201	TACCTGCTTA	ACCTAACCCT	GGCTGTTGTC	ACCATGGCTT	ATGAAGAACA
1251	GAACAGAAAT	GTAGCTGCTG	AGACAGAGGC	CAAGGAGAAA	ATGTTTCAGG

1301 AAGCCCAGCA GCTGTTAAGG GAGGAGAAGG AGGCTCTGGT TGCCATGGGA

1351 ATTGACAGAA GTTCCCTTAA TTCCCTTCAA GCTTCATCCT TTTCCCCGAA

1401 GAAGAGGAAG TTTTTCGGTA GTAAGACAAG AAAGTCCTTC TTTATGAGAG

1451 GGTCCAAGAC GGCCCAAGCC TCAGCGTCTG ATTCAGAGGA CGATGCCTCT

1501 AAAAATCCAC AGCTCCTTGA GCAGACCAAA CGACTGTCCC AGAACTTGCC

1551 AGTGGATCTC TTTGATGAGC ACGTGGACCC CCTCCACAGG CAGAGAGCGC

1601 TGAGCGCTGT CAGTATCTTA ACCATCACCA TACAGGAACA AGAAAAATTC

1651 CAGGAGCCTT GTTTCCCATG TGGGAAAAAT TTGGCCTCTA AGTACCTGGT

1701 GTGGGACTGT AGCCCTCAGT GGCTGTGCAT AAAGAAGGTC CTGCGGACCA

1751 TCATGACGGA TCCCTTTACT GAGCTGGCCA TCACCATCTG CATCATCATC

1801 AATACCGTTT TCTTAGCCGT GGAGCACCAC AACATGGATG ACAACTTAAA

1851 GACCATACTG AAAATAGGAA ACTGGGTTTT CACGGGAATT TTCATAGCGG

1901 AAATGTGTCT CAAGATCATC GCGCTCGACC CTTACCACTA CTTCCGGCAC

1951 GGCTGGAATG TTTTTGACAG CATCGTGGCC CTCCTGAGTC TCGCTGATGT

2001 GCTCTACAAC ACACTGTCTG ATAACAATAG GTCTTTCTTG GCTTCCCTCA

2051 GAGTGCTGAG GGTCTTCAAG TTAGCCAAAT CCTGGCCCAC GTTAAACACT

2101 CTCATTAAGA TCATCGGCCA CTCCGTGGGC GCGCTTGGAA ACCTGACTGT

2151 GGTCCTGACT ATCGTGGTCT TCATCTTTTC TGTGGTGGGC ATGCGGCTCT

2201 TCGGCACCAA GTTTAACAAG ACCGCCTACG CCACCCAGGA GCGGCCCAGG

2251 CGGCGCTGGC ACATGGATAA TTTCTACCAC TCCTTCCTGG TGGTGTTCCG

2301 CATCCTCTGT GGGGAATGGA TCGAGAACAT GTGGGGCTGC ATGCAGGATA

2351 TGGACGGCTC CCCGTTGTGC ATCATTGTCT TTGTCCTGAT AATGGTGATC

2401 GGGAAGCTTG TGGTGCTTAA CCTCTTCATT GCCTTGCTGC TCAATTCCTT

2451 CAGCAATGAG GAGAAGGATG GGAGCCTGGA AGGAGAGACC AGGAAAACCA

2501 AAGTGCAGCT AGCCCTGGAT CGGTTCCGCC GGGCCTTCTC CTTCATGCTG

2551 CACGCTCTTC AGAGTTTTTG TTGCAAGAAA TGCAGGAGGA AAAACTCGCC

2601 AAAGCCAAAA GAGACAACAG AAAGCTTTGC TGGTGAGAAT AAAGACTCAA

2651 TCCTCCCGGA TGCGAGGCCC TGGAAGGAGT ATGATACAGA CATGGCTTTG

2701 TACACTGGAC AGGCCGGGGC TCCGCTGGCC CCACTCGCAG AGGTAGAGGA
2751 CGATGTGGAA TATTGTGGTG AAGGCGGTGC CCTACCCACC TCACAACATA
2801 GTGCTGGAGT TCAGGCCGGT GACCTCCCTC CAGAGACCAA GCAGCTCACT
2851 AGCCCGGATG ACCAAGGGGT TGAAATGGAA GTATTTTCTG AAGAAGATCT
2901 GCATTTAAGC ATACAGAGTC CTCGAAAGAA GTCTGACGCA GTGAGCATGC
2951 TCTCGGAATG CAGCACAATT GACCTGAATG ATATCTTTAG AAATTTACAG
3001 AAAACAGTTT CCCCCAAAAA GCAGCCAGAT AGATGCTTTC CCAAGGGCCT
3051 TAGTTGTCAC TTTCTATGCC ACAAAACAGA CAAGAGAAAG TCCCCCTGGG
3101 TCCTGTGGTG GAACATTCGG AAAACCTGCT ACCAAATCGT GAAGCACAGC
3151 TGGTTTGAGA GTTTCATAAT CTTTGTTATT CTGCTGAGCA GTGGAGCGCT
3201 GATATTTGAA GATGTCAATC TCCCCAGCCG GCCCCAAGTT GAGAAATTAC
3251 TAAGGTGTAC CGATAATATT TTCACATTTA TTTTCCTCCT GGAAATGATC
3301 CTGAAGTGGG TGGCCTTTGG ATTCCGGAGG TATTTCACCA GTGCCTGGTG
3351 CTGGCTTGAT TTCCTCATTG TGGTGGTGTC TGTGCTCAGT CTCATGAATC
3401 TACCAAGCTT GAAGTCCTTC CGGACTCTGC GGGCCCTGAG ACCTCTGCGG
3451 GCGCTGTCCC AGTTTGAAGG AATGAAGGTT GTCGTCTACG CCCTGATCAG
3501 CGCCATACCT GCCATTCTCA ATGTCTTGCT GGTCTGCCTC ATTTTCTGGC
3551 TCGTATTTTG TATCTTGGGA GTAAATTTAT TTTCTGGGAA GTTTGGAAGG
3601 TGCATTAACG GGACAGACAT AAATATGTAT TTGGATTTTA CCGAAGTTCC
3651 GAACCGAAGC CAATGTAACA TTAGTAATTA CTCGTGGAAG GTCCCGCAGG
3701 TCAACTTTGA CAACGTGGGG AATGCCTATC TCGCCCTGCT GCAAGTGGCA
3751 ACCTATAAGG GCTGGCTGGA AATCATGAAT GCTGCTGTCG ATTCCAGAGA
3801 GAAAGACGAG CAGCCGGACT TTGAGGCGAA CCTCTACGCG TATCTCTACT
3851 TTGTGGTTTT TATCATCTTC GGCTCCTTCT TTACCCTGAA CCTCTTTATC
3901 GGTGTTATTA TTGACAACTT CAATCAGCAG CAGAAAAAGT TAGGTGGCCA
3951 AGACATTTTT ATGACAGAAG AACAGAAGAA ATATTACAAT GCAATGAAAA
4001 AGTTAGGAAC CAAGAAACCT CAAAAGCCCA TCCCAAGGCC CCTGAACAAA
4051 TGTCAAGCCT TTGTGTTCGA CCTGGTCACA AGCCAGGTCT TTGACGTCAT

4101 CATTCTGGGT CTTATTGTCT TAAATATGAT TATCATGATG GCTGAATCTG

4151 CCGACCAGCC CAAAGATGTG AAGAAAACCT TTGATATCCT CAACATAGCC

4201 TTCGTGGTCA TCTTTACCAT AGAGTGTCTC ATCAAAGTCT TTGCTTTGAG

4251 GCAACACTAC TTCACCAATG GCTGGAACTT ATTTGATTGT GTGGTCGTGG

4301 TTCTTTCTAT CATTAGTACC CTGGTTTCCC GCTTGGAGGA CAGTGACATT

4351 TCTTTCCCGC CCACGCTCTT CAGAGTCGTC CGCTTGGCTC GGATTGGTCG

4401 AATCCTCAGG CTGGTCCGGG CTGCCCGGGG AATCAGGACC CTCCTCTTTG

4451 CTTTGATGAT GTCTCTCCCC TCTCTCTTCA ACATCGGTCT GCTGCTCTTC

4501 CTGGTGATGT TCATTTACGC CATCTTTGGG ATGAGCTGGT TTTCCAAAGT

4551 GAAGAAGGGC TCCGGGATCG ACGACATCTT CAACTTCGAG ACCTTTACGG

4601 GCAGCATGCT GTGCCTCTTC CAGATAACCA CTTCGGCTGG CTGGGATACC

4651 CTCCTCAACC CCATGCTGGA GGCAAAAGAA CACTGCAACT CCTCCTCCCA

4701 AGACAGCTGT CAGCAGCCGC AGATAGCCGT CGTCTACTTC GTCAGTTACA

4751 TCATCATCTC CTTCCTCATC GTGGTCAACA TGTACATCGC TGTGATCCTC

4801 GAGAACTTCA ACACAGCCAC GGAGGAGAGC GAGGACCCTC TGGGAGAGGA

4851 CGACTTTGAA ATCTTCTATG AGGTCTGGGA GAAGTTTGAC CCCGAGGCGT

4901 CGCAGTTCAT CCAGTATTCG GCCCTCTCTG ACTTTGCGGA CGCCCTGCCG

4951 GAGCCGTTGC GTGTGGCCAA GCCGAATAAG TTTCAGTTTC TAGTGATGGA

5001 CTTGCCCATG GTGATGGGCG ACCGCCTCCA TTGCATGGAT GTTCTCTTTG

5051 CTTTCACTAC CAGGGTCCTC GGGGACTCCA GCGGCTTGGA TACCATGAAA

5101 ACCATGATGG AGGAGAAGTT TATGGAGGCC AACCCTTTTA AGAAGCTCTA

5151 CGAGCCCATA GTCACCACCA CCAAGAGGAA GGAGGAGGAG CAAGGCGCCG

5201 CCGTCATCCA GAGGGCCTAC CGGAAACACA TGGAGAAGAT GGTCAAACTG

5251 AGGCTGAAGG ACAGGTCAAG TTCATCGCAC CAGGTGTTTT GCAATGGAGA

5301 CTTGTCCAGC TTGGATGTGG CCAAGGTCAA GGTTCACAAT GACTGAACCC

5351 TCATCTCCAC CCCTACCTCA CTGCCTCACA GCTTAGCCTC CAGCCTCTGG

5401 CGAGCAGGCG GCAGACTCAC TGAACACAGG CCGTTCGATC TGTGTTTTTG

5451 GCTGAACGAG GTGACAGGTT GGCGTCCATT TTTAAATGAC TCTTGGAAAG

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5501 ATTTCATGTA GAGAGATGTT AGAAGGGACT GCAAAGGACA CCGACCATAA

5551 CGGAAGGCCT GGAGGACAGT CCAACTTACA TAAAGATGAG AAACAAGAAG

5601 GAAAGATCCC AGGAAAACTT CAGATTGTGT TCTCAGTACA TTCCCCAATG

5651 TGTCTGTTCG GTGTTTTGAG TATGTGACCT GCCACATGTA GCTCTTTTTT

5701 GCATGTACGT CAAAACCCTG CAGTAAGTTA ATAGCTTGCT ACGGGTGTTC

5751 CTACCAGCAT CACAGAATTG GGTGTATGAC TCAAACCTAA AAGCATGACT

5801 CTGACTTGTC AGTCAGCACC CCGACTTTCA GACGCTCCAA TCTCTGTCCC

5851 AGGTGTCTAA CGAATAAATA GGTAAAAGAA AAAAAAAAAA AAAAAAA

Figure 3

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-47   GGAGCCATACGGTGCCCTGATCCTCTGTACCAGGAAGACAGGGTGAAGATGGAGGAGAGG   12
1                                                     M  E  E  R     4

13    TACTACCCGGTGATCTTCCCGGACGAGCGGAATTTCCGCCCCTTCACTTCCGACTCTCTG   72
5     Y  Y  P  V  I  F  P  D  E  R  N  F  R  P  F  T  S  D  S  L     24

73    GCTGCCATAAAGAAGCGGATTGCTATCCAAAAGGAGAGGAAGAAGTCCAAAGACAAGGCG   132
25    A  A  I  K  K  R  I  A  I  Q  K  E  R  K  K  S  K  D  K  A     44

133   GCAGCTGAGCCCCAGCCTCGGCCTCAGCTTGACCTAAAGGCCTCCAGGAAGTTACCTAAG   192
45    A  A  E  P  Q  P  R  P  Q  L  D  L  K  A  S  R  K  L  P  K     64

193   CTTTATGGTGACATTCCCCCTGAGCTTGTTACGAAACCTCTGGAGGACCTGGACCCCTAC   252
65    L  Y  G  D  I  P  P  E  L  V  T  K  P  L  E  D  L  D  P  Y     84

253   TACAAAGACCATAAGACATTCATGGTGTTGAACAAGAAAAGAACAATTTATCGCTTCAGC   312
85    Y  K  D  H  K  T  F  M  V  L  N  K  K  R  T  I  Y  R  F  S     104

313   GCCAAGCGGGCCTTGTTCATTCTGGGGCCTTTTAATCCCCTCAGAAGCTTAATGATTCGT   372
105   A  K  R  A  L  F  I  L  G  P  F  N  P  L  R  S  L  M  I  R     124

373   ATCTCTGTCCATTCAGTCTTTAGCATGTTCATCATCTGCACGGTGATCATCAACTGTATG   432
125   I  S  V  H  S  V  F  S  M  F  I  I  C  T  V  I  I  N  C  M     144

433   TTCATGGCGAATTCTATGGAGAGAAGTTTCGACAACGACATTCCCGAATACGTCTTCATT   492
145   F  M  A  N  S  M  E  R  S  F  D  N  D  I  P  E  Y  V  F  I     164

493   GGGATTTATATTTTAGAAGCTGTGATTAAAATATTGGCAAGAGGCTTCATTGTGGATGAG   552
165   G  I  Y  I  L  E  A  V  I  K  I  L  A  R  G  F  I  V  D  E     184

553   TTTTCCTTCCTCCGAGATCCGTGGAACTGGCTGGACTTCATTGTCATTGGAACAGCGATC   612
185   F  S  F  L  R  D  P  W  N  W  L  D  F  I  V  I  G  T  A  I     204

613   GCAACTTGTTTTCCGGGCAGCCAAGTCAATCTTTCAGCTCTTCGTACCTTCCGAGTGTTC   672
205   A  T  C  F  P  G  S  Q  V  N  L  S  A  L  R  T  F  R  V  F     224

673   AGAGCTCTGAAGGCGATTTCAGTTATCTCAGGTCTGAAGGTCATCGTAGGTGCCCTGCTG   732
225   R  A  L  K  A  I  S  V  I  S  G  L  K  V  I  V  G  A  L  L     244

733   CGCTCGGTGAAGAAGCTGGTAGACGTGATGGTCCTCACTCTCTTCTGCCTCAGCATCTTT   792
245   R  S  V  K  K  L  V  D  V  M  V  L  T  L  F  C  L  S  I  F     264

793   GCCCTGGTCGGTCAGCAGCTGTTCATGGGAATTCTGAACCAGAAGTGTATTAAGCACAAC   852
265   A  L  V  G  Q  Q  L  F  M  G  I  L  N  Q  K  C  I  K  H  N     284

853   TGTGGCCCCAACCCTGCATCCAACAAGGATTGCTTTGAAAAGGAAAAAGATAGCGAAGAC   912
285   C  G  P  N  P  A  S  N  K  D  C  F  E  K  E  K  D  S  E  D     304

913   TTCATAATGTGTGGTACCTGGCTCGGCAGCAGACCCTGTCCCAATGGTTCTACGTGCGAT   972
305   F  I  M  C  G  T  W  L  G  S  R  P  C  P  N  G  S  T  C  D     324

973   AAAACCACATTGAACCCAGACAATAATTATACAAAGTTTGACAACTTTGGCTGGTCCTTT   1032
325   K  T  T  L  N  P  D  N  N  Y  T  K  F  D  N  F  G  W  S  F     344

1033  CTCGCCATGTTCCGGGTTATGACTCAAGACTCCTGGGAGAGGCTTTACCGACAGATCCTG   1092
345   L  A  M  F  R  V  M  T  Q  D  S  W  E  R  L  Y  R  Q  I  L     364

1093  CGGACCTCTGGGATCTACTTTGTCTTCTTCTTCGTGGTGGTCATCTTCCTGGGCTCCTTC   1152
365   R  T  S  G  I  Y  F  V  F  F  F  V  V  V  I  F  L  G  S  F     384

1153  TACCTGCTTAACCTAACCCTGGCTGTTGTCACCATGGCTTATGAAGAACAGAACAGAAAT   1212
385   Y  L  L  N  L  T  L  A  V  V  T  M  A  Y  E  E  Q  N  R  N     404
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1213 GTAGCTGCTGAGACAGAGGCCAAGGAGAAAATGTTTCAGGAAGCCCAGCAGCTGTTAAGG 1272
405 V A A E T E A K E K M F Q E A Q Q L L R 424

1273 GAGGAGAAGGAGGCTCTGGTTGCCATGGGAATTGACAGAAGTTCCCTTAATTCCCTTCAA 1332
425 E E K E A L V A M G I D R S S L N S L Q 444

1333 GCTTCATCCTTTTCCCCGAAGAAGAGGAAGTTTTTCGGTAGTAAGACAAGAAAGTCCTTC 1392
445 A S S F S P K K R K F F G S K T R K S F 464

1393 TTTATGAGAGGGTCCAAGACGGCCCAAGCCTCAGCGTCTGATTCAGAGGACGATGCCTCT 1452
465 F M R G S K T A Q A S A S D S E D D A S 484

1453 AAAAATCCACAGCTCCTTGAGCAGACCAAACGACTGTCCCAGAACTTGCCAGTGGATCTC 1512
485 K N P Q L L E Q T K R L S Q N L P V D L 504

1513 TTTGATGAGCACGTGGACCCCCTCCACAGGCAGAGAGCGCTGAGCGCTGTCAGTATCTTA 1572
505 F D E H V D P L H R Q R A L S A V S I L 524

1573 ACCATCACCATACAGGAACAAGAAAAATTCCAGGAGCCTTGTTTCCCATGTGGAAAAAT 1632
525 T I T I Q E Q E K F Q E P C F P C G K N 544

1633 TTGGCCTCTAAGTACCTGGTGTGGGACTGTAGCCCTCAGTGGCTGTGCATAAAGAAGGTC 1692
545 L A S K Y L V W D C S P Q W L C I K K V 564

1693 CTGCGGACCATCATGACGGATCCCTTTACTGAGCTGGCCATCACCATCTGCATCATCATC 1752
565 L R T I M T D P F T E L A I T I C I I I 584

1753 AATACCGTTTTCTTAGCCGTGGAGCACCACAACATGGATGACAACTTAAAGACCATACTG 1812
585 N T V F L A V E H H N M D D N L K T I L 604

1813 AAAATAGGAAACTGGGTTTTCACGGGAATTTTCATAGCGGAAATGTGTCTCAAGATCATC 1872
605 K I G N W V F T G I F I A E M C L K I I 624

1873 GCGCTCGACCCTTACCACTACTTCCGGCACGGCTGGAATGTTTTTGACAGCATCGTGGCC 1932
625 A L D P Y H Y F R H G W N V F D S I V A 644

1933 CTCCTGAGTCTCGCTGATGTGCTCTACAACACACTGTCTGATAACAATAGGTCTTTCTTG 1992
645 L L S L A D V L Y N T L S D N N R S F L 664

1993 GCTTCCCTCAGAGTGCTGAGGGTCTTCAAGTTAGCCAAATCCTGGCCCACGTTAAACACT 2052
665 A S L R V L R V F K L A K S W P T L N T 684

2053 CTCATTAAGATCATCGGCCACTCCGTGGGCGCGCTTGGAAACCTGACTGTGGTCCTGACT 2112
685 L I K I I G H S V G A L G N L T V V L T 704

2113 ATCGTGGTCTTCATCTTTTCTGTGGTGGGCATGCGGCTCTTCGGCACCAAGTTTAACAAG 2172
705 I V V F I F S V V G M R L F G T K F N K 724

2173 ACCGCCTACGCCACCCAGGAGCGGCCCAGGCGGCGCTGGCACATGGATAATTTCTACCAC 2232
725 T A Y A T Q E R P R R R W H M D N F Y H 744

2233 TCCTTCCTGGTGGTGTTCCGCATCCTCTGTGGGGAATGGATCGAGAACATGTGGGGCTGC 2292
745 S F L V V F R I L C G E W I E N M W G C 764

2293 ATGCAGGATATGGACGGCTCCCCGTTGTGCATCATTGTCTTTGTCCTGATAATGGTGATC 2352
765 M Q D M D G S P L C I I V F V L I M V I 784

2353 GGGAAGCTTGTGGTGCTTAACCTCTTCATTGCCTTGCTGCTCAATTCCTTCAGCAATGAG 2412
785 G K L V V L N L F I A L L L N S F S N E 804

2413 GAGAAGGATGGGAGCCTGGAAGGAGAGACCAGGAAAACCAAAGTGCAGCTAGCCCTGGAT 2472
805 E K D G S L E G E T R K T K V Q L A L D 824

2473 CGGTTCCGCCGGGCCTTCTCCTTCATGCTGCACGCTCTTCAGAGTTTTTGTTGCAAGAAA 2532
825 R F R R A F S F M L H A L Q S F C C K K 844

2533 TGCAGGAGGAAAAACTCGCCAAAGCCAAAAGAGACAACAGAAAGCTTTGCTGGTGAGAAT 2592
845 C R R K N S P K P K E T T E S F A G E N 864

2593 AAAGACTCAATCCTCCCGGATGCGAGGCCCTGGAAGGAGTATGATACAGACATGGCTTTG 2652

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865   K   D   S   I   L   P   D   A   R   P   W   K   E   Y   D   T   D   M   A   L      884

2653  TACACTGGACAGGCCGGGGCTCCGCTGGCCCCACTCGCAGAGGTAGAGGACGATGTGGAA   2712
885   Y   T   G   Q   A   G   A   P   L   A   P   L   A   E   V   E   D   D   V   E      904

2713  TATTGTGGTGAAGGCGGTGCCCTACCCACCTCACAACATAGTGCTGGAGTTCAGGCCGGT   2772
905   Y   C   G   E   G   G   A   L   P   T   S   Q   H   S   A   G   V   Q   A   G      924

2773  GACCTCCCTCCAGAGACCAAGCAGCTCACTAGCCCGGATGACCAAGGGGTTGAAATGGAA   2832
925   D   L   P   P   E   T   K   Q   L   T   S   P   D   D   Q   G   V   E   M   E      944

2833  GTATTTTCTGAAGAAGATCTGCATTTAAGCATACAGAGTCCTCGAAAGAAGTCTGACGCA   2892
945   V   F   S   E   E   D   L   H   L   S   I   Q   S   P   R   K   K   S   D   A      964

2893  GTGAGCATGCTCTCGGAATGCAGCACAATTGACCTGAATGATATCTTTAGAAATTTACAG   2952
965   V   S   M   L   S   E   C   S   T   I   D   L   N   D   I   F   R   N   L   Q      984

2953  AAAACAGTTTCCCCCAAAAAGCAGCCAGATAGATGCTTTCCCAAGGGCCTTAGTTGTCAC   3012
985   K   T   V   S   P   K   K   Q   P   D   R   C   F   P   K   G   L   S   C   H      1004

3013  TTTCTATGCCACAAAACAGACAAGAGAAAGTCCCCCTGGGTCCTGTGGTGGAACATTCGG   3072
1005  F   L   C   H   K   T   D   K   R   K   S   P   W   V   L   W   W   N   I   R      1024

3073  AAAACCTGCTACCAAATCGTGAAGCACAGCTGGTTTGAGAGTTTCATAATCTTTGTTATT   3132
1025  K   T   C   Y   Q   I   V   K   H   S   W   F   E   S   F   I   I   F   V   I      1044

3133  CTGCTGAGCAGTGGAGCGCTGATATTTGAAGATGTCAATCTCCCCAGCCGGCCCCAAGTT   3192
1045  L   L   S   S   G   A   L   I   F   E   D   V   N   L   P   S   R   P   Q   V      1064

3193  GAGAAATTACTAAGGTGTACCGATAATATTTTCACATTTATTTTCCTCCTGGAAATGATC   3252
1065  E   K   L   L   R   C   T   D   N   I   F   T   F   I   F   L   L   E   M   I      1084

3253  CTGAAGTGGGTGGCCTTTGGATTCCGGAGGTATTTCACCAGTGCCTGGTGCTGGCTTGAT   3312
1085  L   K   W   V   A   F   G   F   R   R   Y   F   T   S   A   W   C   W   L   D      1104

3313  TTCCTCATTGTGGTGGTGTCTGTGCTCAGTCTCATGAATCTACCAAGCTTGAAGTCCTTC   3372
1105  F   L   I   V   V   V   S   V   L   S   L   M   N   L   P   S   L   K   S   F      1124

3373  CGGACTCTGCGGGCCCTGAGACCTCTGCGGGCGCTGTCCCAGTTTGAAGGAATGAAGGTT   3432
1125  R   T   L   R   A   L   R   P   L   R   A   L   S   Q   F   E   G   M   K   V      1144

3433  GTCGTCTACGCCCTGATCAGCGCCATACCTGCCATTCTCAATGTCTTGCTGGTCTGCCTC   3492
1145  V   V   Y   A   L   I   S   A   I   P   A   I   L   N   V   L   L   V   C   L      1164

3493  ATTTTCTGGCTCGTATTTTGTATCTTGGGAGTAAATTTATTTTCTGGGAAGTTTGGAAGG   3552
1165  I   F   W   L   V   F   C   I   L   G   V   N   L   F   S   G   K   F   G   R      1184

3553  TGCATTAACGGGACAGACATAAATATGTATTTGGATTTTACCGAAGTTCCGAACCGAAGC   3612
1185  C   I   N   G   T   D   I   N   M   Y   L   D   F   T   E   V   P   N   R   S      1204

3613  CAATGTAACATTAGTAATTACTCGTGGAAGGTCCCGCAGGTCAACTTTGACAACGTGGGG   3672
1205  Q   C   N   I   S   N   Y   S   W   K   V   P   Q   V   N   F   D   N   V   G      1224

3673  AATGCCTATCTCGCCCTGCTGCAAGTGGCAACCTATAAGGGCTGGCTGGAAATCATGAAT   3732
1225  N   A   Y   L   A   L   L   Q   V   A   T   Y   K   G   W   L   E   I   M   N      1244

3733  GCTGCTGTCGATTCCAGAGAGAAAGACGAGCAGCCGGACTTTGAGGCGAACCTCTACGCG   3792
1245  A   A   V   D   S   R   E   K   D   E   Q   P   D   F   E   A   N   L   Y   A      1264

3793  TATCTCTACTTTGTGGTTTTTATCATCTTCGGCTCCTTCTTTACCCTGAACCTCTTTATC   3852
1265  Y   L   Y   F   V   V   F   I   I   F   G   S   F   F   T   L   N   L   F   I      1284

3853  GGTGTTATTATTGACAACTTCAATCAGCAGCAGAAAAAGTTAGGTGGCCAAGACATTTTT   3912
1285  G   V   I   I   D   N   F   N   Q   Q   Q   K   K   L   G   G   Q   D   I   F      1304

3913  ATGACAGAAGAACAGAAGAAATATTACAATGCAATGAAAAAGTTAGGAACCAAGAAACCT   3972
1305  M   T   E   E   Q   K   K   Y   Y   N   A   M   K   K   L   G   T   K   K   P      1324

3973  CAAAAGCCCATCCCAAGGCCCCTGAACAAATGTCAAGCCTTTGTGTTCGACCTGGTCACA   4032
1325  Q   K   P   I   P   R   P   L   N   K   C   Q   A   F   V   F   D   L   V   T      1344
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4033 AGCCAGGTCTTTGACGTCATCATTCTGGGTCTTATTGTCTTAAATATGATTATCATGATG 4092

1345 S Q V F D V I I L G L I V L N M I I M M 1364

4093 GCTGAATCTGCCGACCAGCCCAAAGATGTGAAGAAAACCTTTGATATCCTCAACATAGCC 4152

1365 A E S A D Q P K D V K K T F D I L N I A 1384

4153 TTCGTGGTCATCTTTACCATAGAGTGTCTCATCAAAGTCTTTGCTTTGAGGCAACACTAC 4212

1385 F V V I F T I E C L I K V F A L R Q H Y 1404

4213 TTCACCAATGGCTGGAACTTATTTGATTGTGTGGTCGTGGTTCTTTCTATCATTAGTACC 4272

1405 F T N G W N L F D C V V V V L S I I S T 1424

4273 CTGGTTTCCCGCTTGGAGGACAGTGACATTTCTTTCCCGCCCACGCTCTTCAGAGTCGTC 4332

1425 L V S R L E D S D I S F P P T L F R V V 1444

4333 CGCTTGGCTCGGATTGGTCGAATCCTCAGGCTGGTCCGGGCTGCCCGGGGAATCAGGACC 4392

1445 R L A R I G R I L R L V R A A R G I R T 1464

4393 CTCCTCTTTGCTTTGATGATGTCTCTCCCCTCTCTCTTCAACATCGGTCTGCTGCTCTTC 4452

1465 L L F A L M M S L P S L F N I G L L L F 1484

4453 CTGGTGATGTTCATTTACGCCATCTTTGGGATGAGCTGGTTTTCCAAAGTGAAGAAGGGC 4512

1485 L V M F I Y A I F G M S W F S K V K K G 1504

4513 TCCGGGATCGACGACATCTTCAACTTCGAGACCTTTACGGGCAGCATGCTGTGCCTCTTC 4572

1505 S G I D D I F N F E T F T G S M L C L F 1524

4573 CAGATAACCACTTCGGCTGGCTGGGATACCCTCCTCAACCCCATGCTGGAGGCAAAAGAA 4632

1525 Q I T T S A G W D T L L N P M L E A K E 1544

4633 CACTGCAACTCCTCCTCCCAAGACAGCTGTCAGCAGCCGCAGATAGCCGTCGTCTACTTC 4692

1545 H C N S S S Q D S C Q Q P Q I A V V Y F 1564

4693 GTCAGTTACATCATCATCTCCTTCCTCATCGTGGTCAACATGTACATCGCTGTGATCCTC 4752

1565 V S Y I I I S F L I V V N M Y I A V I L 1584

4753 GAGAACTTCAACACAGCCACGGAGGAGAGCGAGGACCCTCTGGGAGAGGACGACTTTGAA 4812

1585 E N F N T A T E E S E D P L G E D D F E 1604

4813 ATCTTCTATGAGGTCTGGGAGAAGTTTGACCCCGAGGCGTCGCAGTTCATCCAGTATTCG 4872

1605 I F Y E V W E K F D P E A S Q F I Q Y S 1624

4873 GCCCTCTCTGACTTTGCGGACGCCCTGCCGGAGCCGTTGCGTGTGGCCAAGCCGAATAAG 4932

1625 A L S D F A D A L P E P L R V A K P N K 1644

4933 TTTCAGTTTCTAGTGATGGACTTGCCCATGGTGATGGGCGACCGCCTCCATTGCATGGAT 4992

1645 F Q F L V M D L P M V M G D R L H C M D 1664

4993 GTTCTCTTTGCTTTCACTACCAGGGTCCTCGGGGACTCCAGCGGCTTGGATACCATGAAA 5052

1665 V L F A F T T R V L G D S S G L D T M K 1684

5053 ACCATGATGGAGGAGAAGTTTATGGAGGCCAACCCTTTTAAGAAGCTCTACGAGCCCATA 5112

1685 T M M E E K F M E A N P F K K L Y E P I 1704

5113 GTCACCACCACCAAGAGGAAGGAGGAGGAGCAAGGCGCCGCCGTCATCCAGAGGGCCTAC 5172

1705 V T T T K R K E E E Q G A A V I Q R A Y 1724

5173 CGGAAACACATGGAGAAGATGGTCAAACTGAGGCTGAAGGACAGGTCAAGTTCATCGCAC 5232

1725 R K H M E K M V K L R L K D R S S S S H 1744

5233 CAGGTGTTTTGCAATGGAGACTTGTCCAGCTTGGATGTGGCCAAGGTCAAGGTTCACAAT 5292

1745 Q V F C N G D L S S L D V A K V K V H N 1764

5293 GACTGAACCCTCATCTCCACCCCTACCTCACTGCCTCACAGCTTAGCCTCCAGCCTCTGG 5352

1765 D * 1766

5353 CGAGCAGGCGGCAGACTCACTGAACACAGGCCGTTCGATCTGTGTTTTTGGCTGAACGAG 5412

5413 GTGACAGGTTGGCGTCCATTTTTAAATGACTCTTGGAAAGATTTCATGTAGAGAGATGTT 5472

5473 AGAAGGGACTGCAAAGGACACCGACCATAACGGAAGGCCTGGAGGACAGTCCAACTTACA 5532

5533 TAAAGATGAGAAACAAGAAGGAAAGATCCCAGGAAAACTTCAGATTGTGTTCTCAGTACA 5592

5593 TTCCCCAATGTGTCTGTTCGGTGTTTTGAGTATGTGACCTGCCACATGTAGCTCTTTTTT 5652

5653 GCATGTACGTCAAAACCCTGCAGTAAGTTAATAGCTTGCTACGGGTGTTCCTACCAGCAT 5712

5713 CACAGAATTGGGTGTATGACTCAAACCTAAAAGCATGACTCTGACTTGTCAGTCAGCACC 5772

5773 CCGACTTTCAGACGCTCCAATCTCTGTCCCAGGTGTCTAACGAATAAATAGGTAAAAGAA 5832

5833 AAAAAAAAAAAAAAAAA 5849

Figure 4

1/2

1 MEERYYPVIF PDERNFRPFT SDSLAAIKKR IAIQKERKKS KDKAAAEPQP
51 RPQLDLKASR KLPKLYGDIP PELVTKPLED LDPYYKDHKT FMVLNKKRTI
101 YRFSAKRALF ILGPFNPLRS LMIRISVHSV FSMFIICLVI INCMEMANSM
151 ESSFDNDIPE YVFTGLYILE AVLKILARGF IVDEFSFLRD PWNWLDFIVI
201 GLAILAICFE SQVNLSALRI FRVFRALKAI SVISGLKVIV GALLRSVKKL
251 VDVMVLTLFC LSIFALVGQQ LFMGILNQKC IKHNCGPNPA SNKDCFEKEK
301 DSEDFIMCGT WLGSRPCPNG STCDKTTLNP DNNYTKFDNF GWSFLAMFRV
351 MTQDSWERLY RQILRTSGLY FVTFFVVVIF LGSFYLLNLI LAVVTMAYEE
401 QNRNVAAETE AKEKMFQEAQ QLLREEKEAL VAMGIDRSSL NSLQASSFSP
451 KKRKFFGSKT RKSFFMRGSK TAQASASDSE DDASKNPQLL EQTKRLSQNL
501 PVDLFDEHVD PLHRQRALSA VSILTITIQE QEKFQEPCFP CGKNLASKYL
551 VWDCSPQWLC IKKVLRTIMI DPFTDLAITI CIILNTVFLA VEHHNMDDNL
601 KTILKYGNWV FIGSFIAEMC LKIIALEPYH YFRHGWNIFD SIVALLSLAD
651 VEYNILSDNN RSFLASLRVL RVFKLAKSWP TLNTLIKIIG HSVGALGNLT
701 VVLTIVVFIF SVVGMRLFGI KFNKTAYATQ ERPRRRWHMD NFYHSFLVVF
751 RILCGEWIEN MWGCMQDMDG SPLCIIVFVL IMVIGKLVVL NLFIALLLNS
801 FSNEEKDGSL EGETRKTKVQ LALDRFRRAF SFMLHALQSF CCKKCRRKNS
851 PKPKETTESF AGENKDSILP DARPWKEYDT DMALYTGQAG APLAPLAEVE
901 DDVEYCGEGG ALPTSQHSAG VQAGDLPPET KQLTSPDDQG VEMEVFSEED
951 LHLSIQSPRK KSDAVSMLSE CSTIDLNDIF RNLQKTVSPK KQPDRCFPKG
1001 LSCHFLCHKT DKRKSPWVLW WNIRKTCYQI VKHSWFESFI IFVILLSSGA
1051 LIFEDVNLPS RPQVEKLERC NIDNLFITFL FSMLLKWVAS GFRRYFLSAW
1101 GWLDFLIVVV SVISLMNLRS LKSPRILRAL RPLRALSRFE GMKVVVYALI
1151 SAIPAILNVL LVCLIFWLIF SILGVNLFSG KFGRCINGTD INMYLDFTEV
1201 PNRSQCNISN YSWKVPQVNF DNVGNAYLAL LQVATYKGWL EIMNAAVDSR
1251 EKDEQPDFEA NLYAVLYFVV IFILFGSFYI NLFIGVIIDN FNQQKKLGG
1301 QDIFMTEEQK KYYNAMKKLG TKKPQKPIPR PLNKCIAFVF DLVISQMFDV
1351 ILLGLIVLNM IIMMAESADQ PKDVKKTFDI LNIAFVVLFT IECLIKVFAL
1401 RQHYFTNGWN LFDCVVVVLS IISILVSRLE DSDISFPPTL FRVVRLARIG
1451 RILRLVRAAR GIRTLLFALM MSLPSLFNIG LLLFLVMFIY AIFGMSWFSK

1501 VKKGSGIDDI FNFETFTGSM LCLFQITTSA GWDTLLNPML EAKEHCNSSS

1551 QDSCQQ[redacted]A TEESEDPLGE

1601 DDFEIFYEVW EKFDPEASQF IQYSALSDFA DALPEPLRVA KPNKFQFLVM

1651 DLPMVMGDRL HCMDVLFAFT TRVLGDSSGL DTMKTMMEEK FMEANPFKKL

1701 YEPIVTTTKR KEEEQGAAVI QRAYRKHMEK MVKLRLKDRS SSSHQVFCNG

1751 DLSSLDVAKV KVHND*

Figure 5 1/12

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                 1        10         20         30
RBI      - - - M E Q T V L V P P G P D S F N F F T R E S L A A I E R R
RBII     - - - M A R S V L V P P G P D S F R F F T R E S L A A I E Q R
RBIII    - - - M A Q A L L V P P G P E S F R L F T R E S L A A I E K R
PN1      - - - - - M A M L P P P G P Q S F V H F T K Q S L A L I E Q R
NACH6    - M R R S A R L L A P P G P D S F K P F T P E S L A N I E R R
SKM1     M A S S S L P N L V P P G P H C L R P F T P E S L A A I E Q R
PN3      - - - - M E L P F A S V G T T N F R R F T P E S L A E I E K Q
CARDIAC  - - - - M A N L L L P R G T S S F R R F T R E S L A A I E K R
SNS2A    - M E E R Y Y P V I F P D E R N F R P F T S D S L A A I K K R
GLIAL    - - - - - - - M L T S P E P K G L V P F T A E S L E L I K N H
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                          40         50         60
RBI      I A E E K A K N P K P - - - D K K D - - D D E N G P K P N S D
RBII     I A E E K A K R P K Q - - - E R K D - E D D E N G P K P N S D
RBIII    A A E E K A K K P K K - - - E - Q D - I D D E N K P K P N S D
PN1      I S E E K A K E H K D - - - E K K D - - D E E E G P K P S S D
NACH6    I A E S K L K K P P K A D G S H R E - D D E D S K P K P N S D
SKM1     A V E E E A R - - - - L Q R N K Q M - E I E E P E R K P R S D
PN3      I A A H R A A K K A R T K H R G Q E - - D K G E K P R P Q L D
CARDIAC  M A E K Q A R G G S A T S Q E S R E G L Q E E E A P R P Q L D
SNS2A    I A I Q K E R K - - - - - - K S K D K A A A E P Q P R P Q L D
GLIAL    I A - - - - - - - - - - - - K K C N E E H E E E D L K P S R D
```

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                          70         80         90
RBI      L E A G K N L P F I Y G D I P P E M V S E P L E D L D P Y Y I
RBII     L E A G K S L P F I Y G D I P P E M V S E P L E D L D P Y Y I
RBIII    L E A G K N L P F I Y G D I P P E M V S E P L E D L D P Y Y V
PN1      L E A G K Q L P F I Y G D I P P G M V S E P L E D L D P Y Y A
NACH6    L E A G K S L P F I Y G D I P Q G L V A V P L E D F D P Y Y L
SKM1     L E A G K N L P L I Y G D P P P E V I G I P L E D L D P Y Y S
PN3      L K A C N Q L P K F Y G E L P A E L V G E P L E D L D P F Y S
CARDIAC  L Q A S K K L P D L Y G N P P R E L I G E P L E D L D P F Y S
SNS2A    L K A S R K L P K L Y G D I P P E L V T K P L E D L D P Y Y K
GLIAL    I E A G K K L P F A Y G T L P Q G T V S E P L E D V D P Y Y Y
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                        100        110        120
RBI      N K K - T F I V L N K G K A I F R F S A T S A L Y I L T P F N
RBII     N K K - T F I V L N K G K A I S R F S A T S A L Y I L T P F N
RBIII    S K K - T F V V L N K G K A I F R F S A T S A L Y I L T P L N
PN1      D K K - T F I V L N K G K A L F R F N A T P A L Y M L S P F S
NACH6    T Q K - T F V V L N R G K T L F R F S A T P A L Y I L S P F N
SKM1     D K K - T F I V L N K G K A I F R F S A T P A L Y L L S P F S
PN3      T H R - T F M V L N K S R T I S R F S A T W A L W L F S P F N
CARDIAC  T Q K - T F I V L N K G K T I F R F S A T N A L Y V L S P F H
SNS2A    D H K - T F M V L N K K R T I Y R F S A K R A L F I L G P F N
GLIAL    V K R N T F M V L N R N R V I F R F N A V S I L C T L S P L S
```

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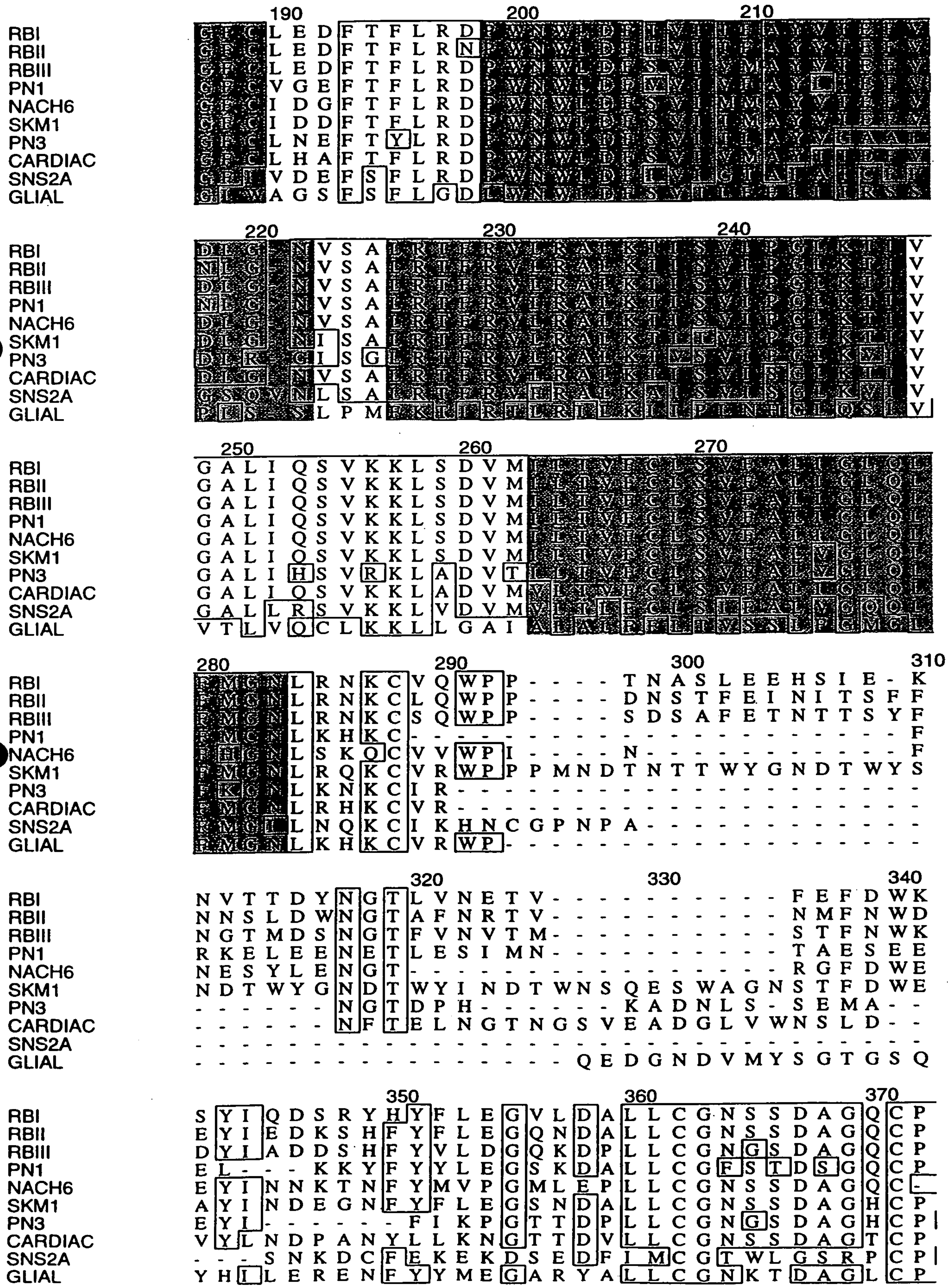
                   130        140        150
RBI      P L R K I A I K I L V H S L F S M L I M C T I L T N C V F M I
RBII     P I R K L A I K I L V H S L F N V L I M C T I L T N C V F M T
RBIII    P V R K I A I K I L V H S L F S M L I M C T I L T N C V F M I
PN1      P L R R I S I K I L V H S L F S M L I M C T I L T N C I F M I
NACH6    L I R R I A I K I L I H S V F S M I I M C T I L T N C V F M T
SKM1     I V R R V A I K V L L H A L F S M F I M I T I L L N C V F M I
PN3      L I R R T A I K V S V H S W F S I F I I I T I L V N C V C M I
CARDIAC  P V R R A A V K I L V H S L F S M L I M C T I L T N C V F M A
SNS2A    P L R S L M I R I S V H S V F S M F I I C L V I I N C M F M A
GLIAL    S L R R A V I K V L V H P L I R L L I L I S V L T D S I L M C
```

```
                 160        170        180
RBI      M S N P P D W T K N V - E Y T F T G I Y T F E S L I K I L A R
RBII     M S N P P D W T K N V - E Y T F T G I Y T F E S L I K I L A R
RBIII    L S N P P D W T K N V - E Y T F T G I Y T F E S L I K I L A R
PN1      L S N P P E W T K N V - E Y T F T G I Y T F E S L I K I L A R
NACH6    F S N P P E W S K N V - E Y T F T G I Y T F E S L V K I I A R
SKM1     M S N L P S W S K H V - E Y T F T G I Y T F E S L I K M L A R
PN3      R T D L P - - - E K V - E Y V F T V I Y T F E A L I K I L A R
CARDIAC  Q H D P P P W T K Y V - E Y T F T A I Y T F E S L V K I L A R
SNS2A    N S M E R S F D N D L P E Y V F L G I Y I L E A V I K I L A R
GLIAL    M S N L P E W I L A V - E N I C L G I Y I L E I L V K V I A R
```


RBI
RBII
RBIII
PN1
NACH6
SKM1
PN3
CARDIAC
SNS2A
GLIAL
190
200
210
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240
250
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350
360
370

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                           380                           390                           400
RBI       E G Y M C V K A G R N P N Y G Y T S F D T F S W A F L S L F R
RBII      E G Y I C V K A G R N P N Y G Y T S F D T F S W A F L S L F R
RBIII     E G Y I C V K A G R N P N Y G Y T S F D T F S W A F L S L F R
PN1       E G Y I C V K A G R N P D Y G Y T S F D T F S W A F L A L F R
NACH6     E G F Q C S K A G R N P N Y G Y T S F D T F S W A F L A L F R
SKM1      E G Y E C I K A G R N P N Y G Y T S Y D T F S W A F L A L F R
PN3       G G Y V C L K T P D N P D F N Y T S F D S F A W A F L S L F R
CARDIAC   E G Y R C L K A G E N P D H G Y T S F D S F A W A F L A L F R
SNS2A     N G S T C D K T T L N P D N N Y T K F D N F G W S F L A M F R
GLIAL     E G Y M C V K E G S N P D N G F T S F D N F G W A L L A M F R

                         410                       420                     430
RBI       L M T Q D F W E N L Y Q L T L R A A G K T Y M I F F V L V I F
RBII      L M T Q D F W E N L Y Q L T L R A A G K T Y M I F F V L V I F
RBIII     L M T Q D Y W E N L Y Q L T L R A A G K T Y M I F F V L V I F
PN1       L M T Q D Y W E N L Y Q Q T L R A A G K T Y M I F F V V V I F
NACH6     L M T Q D Y W E N L Y Q L T L R A A G K T Y M I F F V L V I F
SKM1      L M T Q D Y W E N L F Q L T L R A A G K L Y M I F F V V L I F
PN3       L M T Q D S W E R L Y Q Q T L R A S G K M Y M V F F V L V I F
CARDIAC   L M T Q D C W E R L Y Q Q T L R S A G K I Y M I F F M L V I F
SNS2A     V M T Q D S W E R L Y R Q I L R T S G L Y T F V F F V V V L F
GLIAL     L M T Q D Y P E L L Y H Q I L Y A S G K I Y M I F F V L I S F

                       440                       450                     460
RBI       L G S F Y L L N L I L A V V A M A Y E E Q N Q A T L E E A E Q
RBII      L G S F Y L I N L I L A V V A M A Y E E Q N Q A T L E E A E Q
RBIII     L G S F Y L V N L I L A V V A M A Y E E Q N Q A T L E E A E Q
PN1       L G S F Y L I N L I L A V V A M A Y E E Q N Q A N I E E A K Q
NACH6     V G S F Y P V N L I L A V V A M A Y E E Q N Q A T L E E A E Q
SKM1      L G S F Y L I N L I L A V V A M A Y A E Q N E A T L A E D Q E
PN3       L G S F Y L V N L I L A V V T M A Y E E Q S Q A T I A E I E A
CARDIAC   L G S F Y L V N L I L A V V A M A Y E E Q N Q A T I A E T E E
SNS2A     L G S F Y L L N L T L A V V T M A Y E E Q N R N V A A E T E A
GLIAL     W E A F Y M A S L F L G I I T M A Y E Q E K Q R A S E E S R D

                      470                       480                       490
RBI       K E A E F Q Q M L E Q L K K Q Q E A A Q Q A A A A T A - - - -
RBII      K E A E F Q Q M L E Q L K K Q Q E E A - Q A A A A A A - - - -
RBIII     K E A E F Q Q M L E Q L K K Q Q E E A Q - - A V A A A - - - -
PN1       K E L E F Q Q M L D R L K K E Q E E A E A I A A A A A - - - -
NACH6     K E A E F K A M L E Q L K K Q Q E E A Q A A A M A T S A G T V
SKM1      K E E E F Q Q M L E K Y K K H Q E E L E K A K A A Q A - - - -
PN3       K E K K F Q E A L E V L Q K E Q E V L A A L - - - - - - - - -
CARDIAC   K E K R F Q E A M E M L K K E H E A L T I R - - - - - - - - -
SNS2A     K E K M F Q E A Q Q L L R E E K E A L V A M - - - - - - - - -
GLIAL     M D S K C H Q T V K E F E E E H E G A E L Q C I W F Y E E V L

              500                       510                       520
RBI       S E H S R E P S A A G R L - - - S D S S S E A S K L S S K S A
RBII      S A E S R D F S G A G G I G V F S E S S S V A S K L S S K S E
RBIII     S A A S R D F S G I G G L G E L L E S S S E A S K L S S K S A
PN1       E F T S - - - I G R S R I M G L S E S S S E T S R L S S K S A
NACH6     S E D A I E E E G E D G V G S - P R S S S E L S K L S S K S A
SKM1      - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3       - - - - - - - - - - - - G I D T T S L Q S H S G S P L A S K N A
CARDIAC   - - - - - - - - - - - - G V D T V S R S S L E M S P L A P V T N
SNS2A     - - - - - - - - - - - - G I D R S S L N S L Q A S S F S P K K R
GLIAL     D - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

              530                       540                       550
RBI       K E R R N R R K K R K Q K E Q - S G G E E K D D D E F H K S E
RBII      K E L K N R R K K K K Q K E Q - A G E E E K - E D A V R K S A
RBIII     K E W R N R R K K R R Q R E H L E G N H R A D G D R F P K S E
PN1       K E R R N R R K K K K Q K - M S S G E E K G D D E K L S K S G
NACH6     K E R R N R R K K R K Q K E L S E G E E K G D P E K V F K S E
SKM1      - - - - - - - - - - - - - - - - L E S G E E A D G D P T H N K -
PN3       N E R R P R V K S R - - - - V S E G S T D - - D N R S P Q S D
CARDIAC   H E R K S K R R K R - - - - L S S G T E D G G D D R L P K S D
SNS2A     K F F G S K T R K S - - - - - - - - - - - - - - - - - - - - -
GLIAL     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
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                560                 570                 580
RBI      S E D S I R R K G F R F S I E G N R L T Y E K R Y S S P H Q S
RBII     S E D S I R K K G F Q F S L E G S R L T Y E K R F S S P H Q S
RBIII    S E D S V K R R S F L L S L D G N P L T G D K K L C S P H Q S
PN1      S E E S I R K K S F H L G V E G H H R T R E K R L S T P N Q S
NACH6    S E Y G M R R K A F R - - L P D N R I - - G R K F S I M N Q S
SKM1     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3      P Y N - Q R R M S F L G L S S G - - - - - - R - - - - - - - - -
CARDIAC  S E D G P R A L N Q L S L T H G L S R T S M R - - - - - - - - -
SNS2A    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
GLIAL    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

         590                 600                 610                 620
RBI      L L S I R G S L F S P R R N S R T S L F S F R G - R A K D V G
RBII     L L S I R G S L F S P R R N S R A S L F N F K G - R V K D I G
RBIII    L L S I R G S L F S P R R N S K T S I F S F R G - R A K D V G
PN1      P L S I R G S L F S A R R S S R T S L F S F K G - R G R D L G
NACH6    L L S I P G S P F L S R H N S K S S I F S F G D P S V R D P G
SKM1     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3      - - - - - - - - - - - R R A S H G S V F H F R - - A P S Q D I
CARDIAC  - - - - - - - - - - - P R S S R G S I F T F R - - R R D Q G -
SNS2A    - - - - - - - - - - - - - - - - - - - F F M R G S K T A Q A S
GLIAL    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                             630                 640                 650
RBI      S E N D F A D D E H S T F E D N E S R R D S L F V P R R H G E
RBII     S E N D F A D D E H S T F E D N D S R R D S L F V P H R H G E
RBIII    S E N D F A D D E H S T F E D S E S R R D S L F V P H R P G E
PN1      S E T E F A D D E H S I F G D N E S R R G S L F V P H R P R E
NACH6    S E N E F A D D E H S T V E E S E G R R D S L F I P I R A R E
SKM1     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3      S F P D G I T D D G V F H G D Q E S R R G S I L - - - - L G R
CARDIAC  S E A D F A D D E N S T A G E S E S H R T S L L V P W P L R H
SNS2A    A - - - - - - - - - S D S E D D A S K N P Q L L - - - - - - -
GLIAL    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                           660                 670                 680
RBI      R R N - - - - - S N L S Q T S R S S R M L A G L P A N G K M H
RBII     R R P - - - - - S N V S Q A S R A S R G I P T L P M N G K M H
RBIII    R R N - - - - - S N - - - - - - - - - - - - - - - - - - - - -
PN1      R R S - - - - - S N I S Q A S R S P - - - P V L P V N G K M H
NACH6    R R S S Y S G Y S G Y S Q C S R S S R I S P A C - A Q R E A N
SKM1     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3      G A G Q T G P L P R S P - - - - - - - - - L P Q S P N P G R R H
CARDIAC  P S A Q G Q P G P G A S - - - - - - - - - A P G Y V L N G K R N
SNS2A    - - - - - - - - - - - - E Q T K R L S Q N L P V D L F D E H V -
GLIAL    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

                             690                 700                 710
RBI      S T V D C N G V V S L V G G P S V P T S P V G Q L L P E V I I
RBII     S A V D C N G V V S L V G G P S A L T S P V G Q L L P E - - -
RBIII    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN1      S A V D C N G V V S L V D G P S A L M L P N G Q L L P E V I I
NACH6    S T V D C N G V V S L I G - - - - P G S H I G R L L L R Q R L
SKM1     - - - D C N G - - - - - - - - - - - - - - - - - - - - - - - -
PN3      G E E G Q L G V P T - - - - G E L T A G A P E G P A L - - - -
CARDIAC  S T V D C N G V V S L L G A G D A E A T S P G S Y L L R P M V
SNS2A    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
GLIAL    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - L L

                       720                 730                 740
RBI      D K P A T D D N G T T T E T E M R K R R S S S F H V S M D F L
RBII     - - - - - - - - G T T T E T E I R K R R S S S Y H V S M D L L
RBIII    - - - - - - - - G T T T E T E V R K R R L S S Y Q I S M E M L
PN1      D K A T S D D S G T T N Q M R - K K R L S S S Y F L S E D M L
NACH6    R W - - - - - - - - - - K L R R K A L D S F S F Y G P T R L L
SKM1     - - - - - - - - - - - - - - - - - - - - - - - - - - S L D A S
PN3      - - - - - D T T - - - - - - - G Q K S F L S - - - - - A G Y L
CARDIAC  L D R P P D T T - T P S E E P G G P Q M L T P Q A P C A D G F
SNS2A    - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
GLIAL    G L E L C I K E M E T T Q I E M K K R S P T S I N T T L D I L
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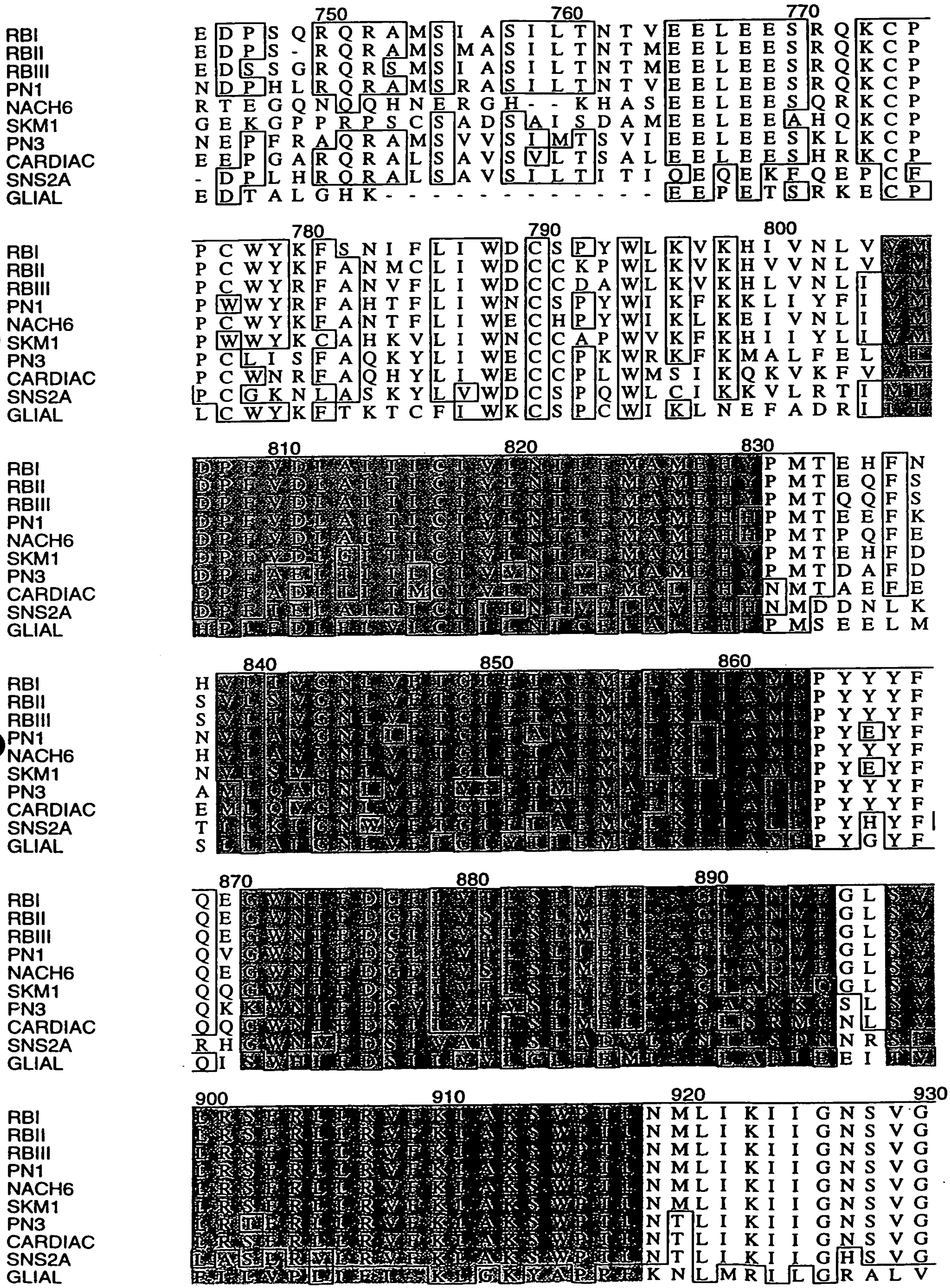
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RBII
RBIII
PN1
NACH6
SKM1
PN3
CARDIAC
SNS2A
GLIAL
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790
800
810
820
830
840
850
860
870
880
890
900
910
920
930

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                                940                           950                     960
RBI       A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K D C
RBII      A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K E C
RBIII     A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K E C
PN1       A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K E C
NACH6     A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K E C
SKM1      A L G N L T L V L A I I V F I F A V V G M Q L F G K S Y K E C
PN3       A L G N L T F I L A I I V F I F A L V G K Q L L S E D Y C C R
CARDIAC   A L G N L T L V L A I I V F I F A V V G M Q L F G K N Y S E L
SNS2A     A L G N L T V V L T I V V F I F S V V G M R L F G T K F N K T
GLIAL     A L K D L V L I V S L F I Y F S A V F G M K L F G R S Y K D C
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                          970                  980                    990
RBI       V C K I A T - D C K L P R W H M N D F F H S F L I V F R V L C
RBII      V C K I S N - D C E L P R W H M H H F F H S F L I V F R V L C
RBIII     V C K I N V - D C K L P R W H M N D F F H S F L I V F R V L C
PN1       V C K I N V - D C K L P R W H M N D F F H S F L I V F R V L C
NACH6     V C K I N Q - E C K L P R W H M N D F F H S F L I V F R V L C
SKM1      V C K I A S - D C N L P R W H M N D F F H S F L I V F R I L C
PN3       K D G V S V W N G E K L R W H M C D F F H S F L V V F R I L C
CARDIAC   R H R I S D - S G L L P R W H M M D F F H A F L I I F R I L C
SNS2A     A - - Y A T Q E R P R R R W H M D N F Y H S F L V V F R I L C
GLIAL     V C H V D - Q D C Q R Q R W H M S D F L H A Y V T V F R I L C
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                               1000                1010                  1020
RBI       G E WI E T M W D C M - E V A G Q A M C L I V F M M V M V I R
RBII      G E WI E T M W D C M - E V A G Q T M C L T V F M M V M V I G
RBIII     G E WI E T M W D C M - E V A G Q T M C L I V F M L V M V I G
PN1       G E WI E T M W D C M - E V A G Q T M C L I V Y M M V M V I G
NACH6     G E WI E T M W D C M - E V A G Q A M C L I V F M M V M V I G
SKM1      G E WI E T M W D C M - E V A G Q A M C L T V F L M V M V L G
PN3       G E WI E N M W V C M - E V S Q K S I C L I L F L I V M V L G
CARDIAC   G E WI E T M W D C M - E V S G Q S L C L I V F L L V M V I G
SNS2A     G E WI E N M W G C M Q D M D G S P L C I I V I V L I M V I G
GLIAL     G E WI E T L W E C M - E V A G E A W C I P F Y M M V I L L G
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                         1030                1040                 1050
RBI       N L V V L N L F L A L L L S S F S A D N L - A A T D D D N E M
RBII      N L V V L N L F L A L L L S S F S S D N L - A A T D D D N E M
RBIII     N L V V L N L F L A L L L S S F S S D N L - A A T D D D N E M
PN1       N L V V L N L F L A L L L S S F S S D N L - T A I E E D T D A
NACH6     N L V V L N L F L A L L L S S F S A D N L - A A T D D D G E M
SKM1      N L V V L N L F L A L I L S S F S A D S L - A A S D E D G E M
PN3       N L V V L N L F L A L L L N S F S A D N L - T A P E D D G E V
CARDIAC   N L V V L N L F L A L L L S S F S A D N L - T A P D E D G E M
SNS2A     K L V V L N L F L A L L L N S F S N E E K D G S L E G E T R K
GLIAL     N L L I L Y L F V G L Y S S F A S Y D - - - A T T E V S K E A
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                     1060                1070                 1080
RBI       N N L Q I A V D R M H K G V A Y V K R K I Y E F I Q Q S F V R
RBII      N N L Q I A V G R M Q K G I D F V K R K I R E F I Q K A F V R
RBIII     N N L Q I A V G R M Q K G I D F V K N K I R E C F R K A F F R
PN1       N N L Q I A V A R I K R G I N Y V K Q T L R E F I L K S F S K
NACH6     N N L Q I S V I R I K K G V A W T K V K V H A F M Q A H F - -
SKM1      N N L Q I A I G R I K W G I G F A K T F L L G L L R G K I L S
PN3       N N L Q L A L A R I Q V L G H R A S R A I A S Y I S S H C R F
CARDIAC   N N L Q L A L A R I Q R G L R F V K R T T W D F C C G I L R -
SNS2A     T K V Q L A L D R F R R A F S F M L H A L Q S F C C K K C R R
GLIAL     K N L Q L A V A W I K M V I N C V L L K I - - - - - - - - - -
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                    1090                1100                 1110
RBI       K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
RBII      K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
RBIII     K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN1       K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
NACH6     K - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
SKM1      P - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
PN3       R W P K V E T Q L G M K P P L T S S E A K N H I A T D A V S A
CARDIAC   R R P K K P A A L A T H S Q L P S C I T A P R S P P P P E V E
SNS2A     K N S P K P K E T T E S F A G E N K D S I L P D A R P W K E Y
GLIAL     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
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                1120                1130                1140
RBI      - - - - - - - Q K I L D E I K P L D D L N N R K D N C T S N H T
RBII     - - - - - - - Q K A L D E I K P L E D L N N K K D S C I S N H T
RBIII    - - - - - - - P K V I - E I Q E - - - - G N K I D S C M S N N T
PN1      - - - - - - - P K G S K D T K R T A D P N N K K E N Y I S N R T
NACH6    - - - - - - - Q R E A D E V K P L D E L Y E K K A N C I A N H T
SKM1     - - - - - - - K E I I L S L G E P G G A G E N A E E S T P E D E
PN3      A V G N L T K P A L S S P K E N H G D F I T D P N V W V S V P
CARDIAC  K V P P A R K E T R F E E D K R P G Q G T P G D S E P V C V P
SNS2A    D T D M A L - - - - - - - - - - - - - Y T G Q A G A P L A P
GLIAL    - - - L C K E K T V S T E A T D Q T C D P S V K E N I S G H T
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           1150                1160                1170
RBI      T - E I G K D L D C L K D V N G T T S G I G T G S S V E K Y I
RBII     T I E I G K D L N Y L K D G N G T T S G I - - G S S V E K Y V
RBIII    G I E I S K E L N Y L K D G N G T T S G V G T G S S V E K Y V
PN1      L A E M S K D H N F L K E K D R I S - - - G Y G S S L D K S F
NACH6    G V D I H R N G D F Q K N G N G T T S G I - - G S S V E K Y I
SKM1     K K E P P P E D K E L K D - N H I L N H V G L T D G P R S S I
PN3      I A E G E S D L D E L E E D M E Q A S Q S S W Q E E D P K G Q
CARDIAC  I A V A E S D T E D Q E E D - E E N S L G T E E E S S K Q E S
SNS2A    L A E V E D D V E Y C G E G G A L P T S Q H S A G V Q A G D L
GLIAL    L S E L S N T Q T F L R Y K D Q - - - - - - - S S G T E K T P
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          1180                1190                1200
RBI      I D E S D Y M S F I N N P S L T V T V P I A V G E S D F E N L
RBII     V D E S D Y M S F I N N P S L T V T V P I A L G E S D F E N L
RBIII    I D E N D Y M S F I N N P S L T V T V P I A V G E S D F E N L
PN1      M D E N D Y Q S F I H N P S L T V T V P I A P G E S D L E I M
NACH6    I D E - D H M S F I N N P N L T V R V P I A V G E S D F E N L
SKM1     - - E L D H L N F I N N P Y L T I Q V P I A S E E S D L E M P
PN3      Q E Q L P Q V Q K C E N H Q A A R S P A S M M S S E D L A P Y
CARDIAC  Q V V S G G H E P Y Q E P R A W S Q V S E T T S S E A G A S T
SNS2A    P P E T K Q L T S P D D Q G V E M E V - - - F S E E D L H - -
GLIAL    V T E S E S Q S L I A S P S V S E T V P I A S G E S D I E N L
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         1210                1220                1230                1240
RBI      N T E D F S S E S D L E E S K E K L N E - - S S S S S E G S T
RBII     N T E E F S S E S D M E E S K E K L N - - - A T S S S E G S T
RBIII    N T E E F S S E S E L E E S K E K L N - - - A T S S S E G S T
PN1      N T E E L S S D S D S D Y S K E K R N R - - - S S S S E C S T
NACH6    N T E D V S S E S D P E G S K D K L D D - - - T S S S E G S T
SKM1     T E E E T D A F S E P E D I K K P L Q P L Y D G N S S V C S T
PN3      L G E S W - - K R K D S P Q V P A E G - V D D T S S S E G S T
CARDIAC  S Q A D W Q Q E Q K T E P Q A P G C G E T P E D S Y S E G S T
SNS2A    - - - - - - - - - - - L S I Q S P R K K S D A V S M L S E C S T
GLIAL    D N K E T R S K S A N G S S K E K M K Q - - - S S S S E C S T
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                        1250                1260                1270
RBI      V D I G A P A E - - E Q P V M E P E E T L E P E A C F T E G C
RBII     V D I G A P A E G - E Q P E A E P E E S L E P E A C F T E D C
RBIII    V D V A P P R E G - E Q A E I E P E E D L K P E A C F T D G C
PN1      V D N P L P G E - - E E A E A E P V N A D E P E A C F T D G C
NACH6    I D I - K P E V E - E V P V E Q P E E Y L D P D A C F T E G C
SKM1     A D Y K P P E E D P E E Q A E E N P E G E Q P E E C F T E A C
PN3      V D C P D P E E I L R K I P E L A D D L D E P D D C F T E G C
CARDIAC  A D M T N T A D L L E Q I P D L G E D V K D P E D C F T E G C
SNS2A    I D L N D I F R N L Q K T V S P K K - - - Q P D R C F P K G L
GLIAL    V D I A I S E E - - - - - - - E E M V Y E H E K S K L H K N G Y
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                     1280                1290                1300
RBI      V Q R F K C C Q I S V E E G R G K Q W W N L R R T C F R I V E
RBII     V R K F K C C Q I S I E E G K G K L W W N L R K T C Y K I V E
RBIII    I K K F P F C Q V S T E E G K G K I W W N L R K T C Y S I V E
PN1      V R R F P C C Q V N V D S G K G K V W W T I R K T C Y R I V E
NACH6    V Q R F K C C Q V N I E E G L G K S W W I L R K T C F L I V E
SKM1     V K R C P C L Y V D I S Q G R G K M W W T L R R A C F K I V E
PN3      T R R C P C C N V N T S K S P W A T G W Q V R K T C Y R I V E
CARDIAC  V R R C P C C M V D T T Q S P G K V W W R L R K T C Y R I V E
SNS2A    S C H F L C H K T D K R K S P W V L W W N I R K T C Y Q L V K
GLIAL    E R K S S A G Q V S R E S R N G K I W R N I R K T C K I V E
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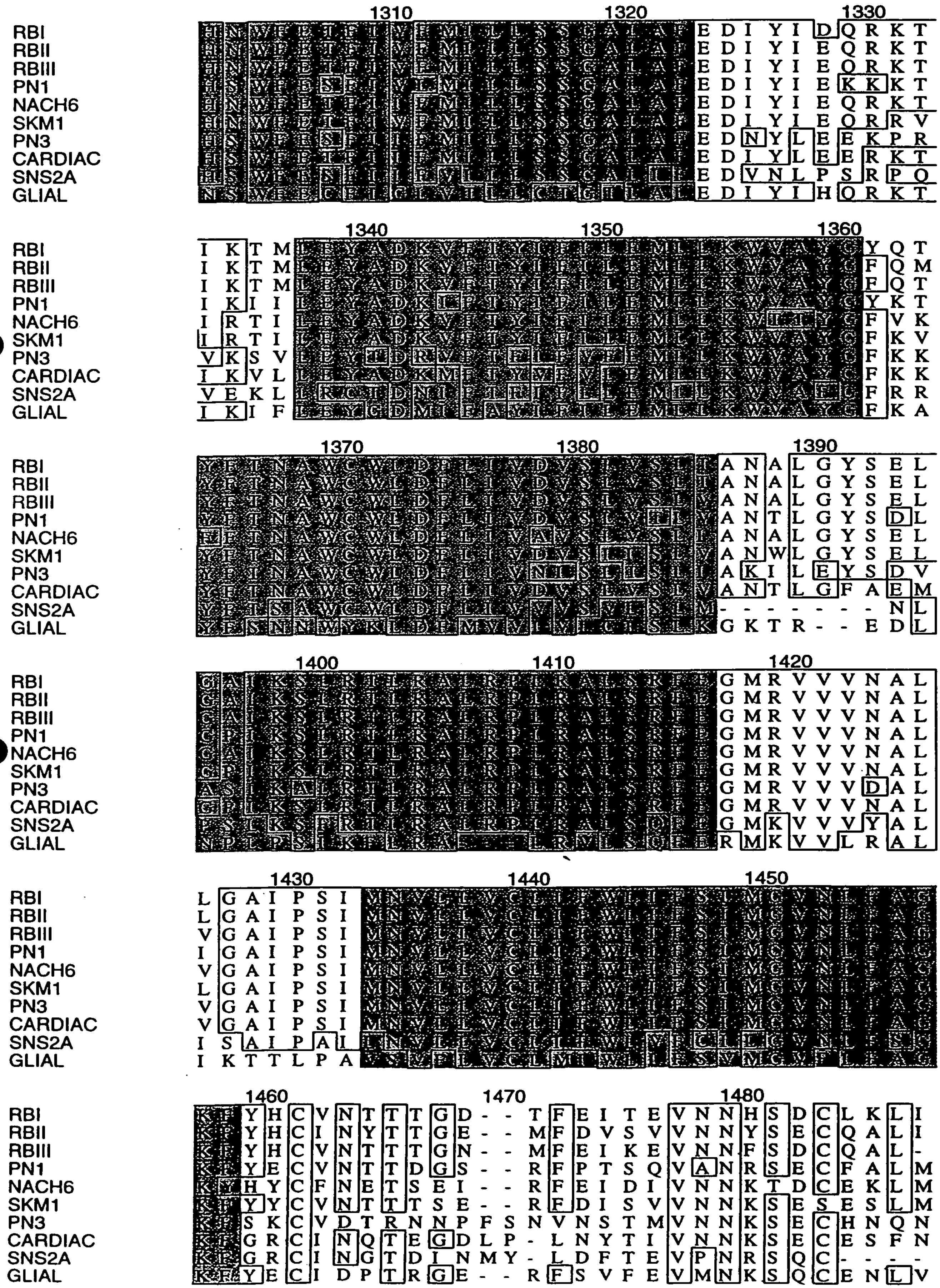
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PN3
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SNS2A
GLIAL

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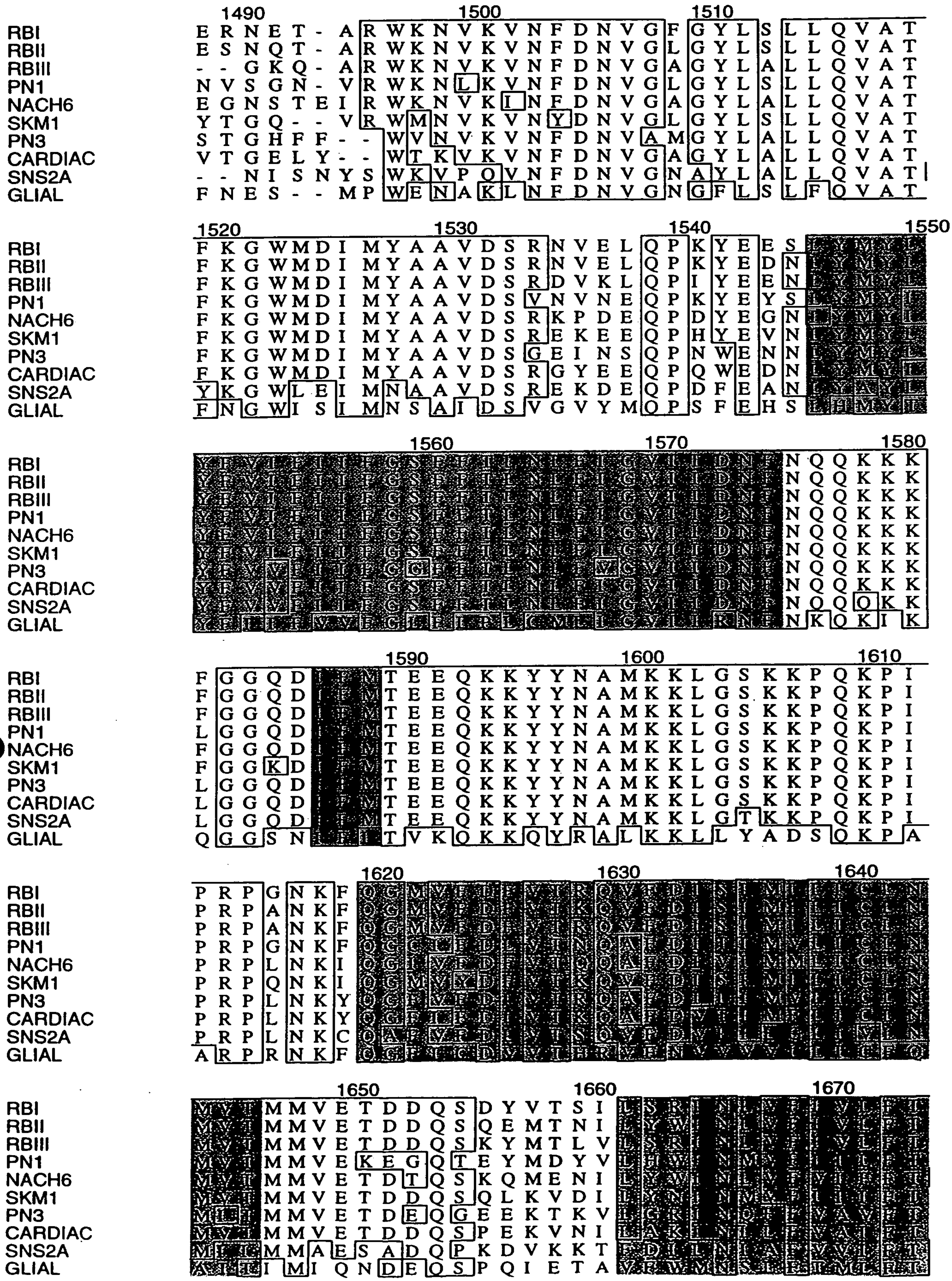
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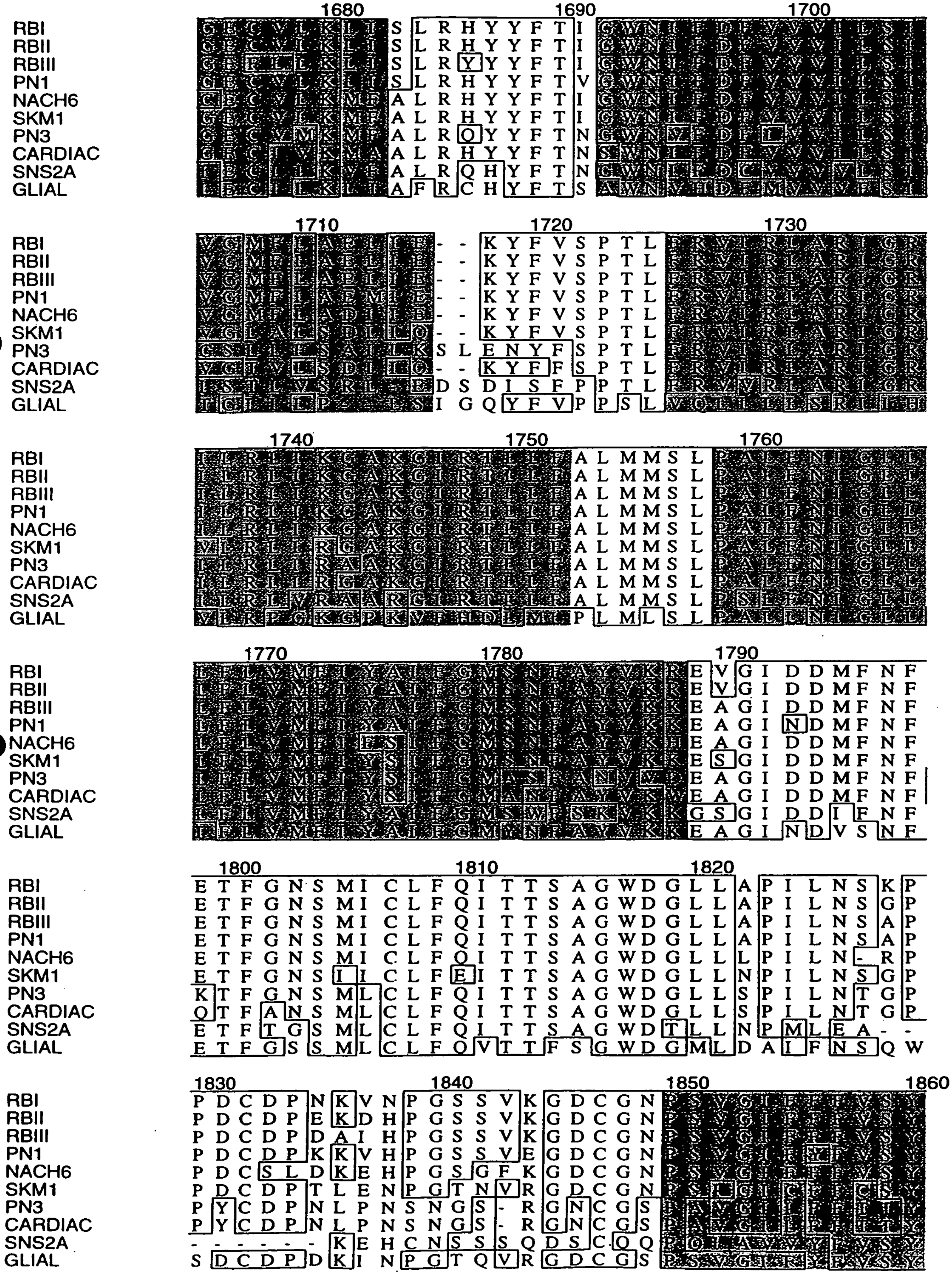
PN1
NACH6
SKM1
PN3
CARDIAC
SNS2A
GLIAL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1850
1860
RBI
RBII
RBIII
PN1
NACH6
SKM1
PN3
CARDIAC
SNS2A
GLIAL

	1870 · 1880 · 1890
RBI	I I I S F L V V V N M Y I A V I L E N F S V A T E E S A E P L
RBII	I I I S F L V V V N M Y I A V I L E N F S V A T E E S A E P L
RBIII	I I I S F L V V V N M Y I A V I L E N F S V A T E E S A E P L
PN1	I I I S F L V V V N M Y I A V I L E N F S V A T E E S T E P L
NACH6	I I I S F L I V V N M C I A I I L E N F S V A T E E S A D P L
SKM1	I I I S F L I V V N M Y I A L I L E N F N V A T E E S S E P L
PN3	I I I S F L I V V N M Y I A V I L E N F N V A T E E S T E P L
CARDIAC	I I I S F L I V V N M Y I A L I L E N F S V A T E E S T E P L
SNS2A	I I I S F L I V V N M Y I A V I L E N F N T A T E E S E D P L
GLIAL	L L I S W L L I V N M Y V V L I M E F L S I P S K R K N R T L

	1900 · 1910 · 1920
RBI	S E D D F E M F Y E V W E K F D P D A T Q F M E F E K L S Q F
RBII	S E D D F E M F Y E V W E K F D P D A T Q F I E F C K L S D F
RBIII	S E D D F E M F Y E V W E K F D P D A T Q F I E F C K L S D F
PN1	S E D D F E M F Y E V W E K F D P D A T Q F I E F C K L S D F
NACH6	S E D D F E T F Y E I W E K F D P D A T Q F I E Y C K L A D F
SKM1	S E D D F E M F Y E T W E K F D P D A T Q F I D Y S R L S D F
PN3	S E D D F D M F Y E T W E K F D P E A T Q F I A F S A L S D F
CARDIAC	S E D D F D M F Y E I W E K F D P E A T Q F I E Y L A L S D F
SNS2A	G E D D F E I F Y E V W E K F D P E A S Q F I Q Y S A L S D F
GLIAL	S E D D F R R F F K V W N R F D P D R T Q Y I D S T K L S D F

	1930 · 1940 · 1950
RBI	A A A L E P P L N L P Q P N K L Q L I A M D L P M V S G D R I
RBII	A A A L D P P L L I A K P N K V Q L I A M D L P M V S G D R I
RBIII	A A A L D P P L L I A K P N K V Q L I A M D L P M V S G D R I
PN1	A A A L D P P L L I A K P N K V Q L I A M D L P M V S G D R I
NACH6	A D A L E H P L R V P K P N T I E L I A M D L P M V S G D R I
SKM1	V D T L Q E P L K I A K P N K I K L I T L D L P M V P G D K I
PN3	A D T L S G P L R I P K P N Q N I L I Q M D L P L V P G D K I
CARDIAC	A D A L S E P L R I A K P N Q I S L I N M D L P M V S G D R I
SNS2A	A D A L P E P L R V A K P N K F Q F L V M D L P M V M G D R L
GLIAL	A A A L D P P L F M A K P N K G Q L V A M D L P M A A G D R I

	1960 · 1970 · 1980
RBI	H C L D I L F A F T K R V L G E S G E M D A L R I Q M E E R F
RBII	H C L D I L F A F T K R V L G E S G E M D A L R I Q M E E R F
RBIII	H C L D I L F A F T K R V L G E S G E M D A L R I Q M E D R F
PN1	H C L D I L F A F T K R V L G E G G E M D S L R S Q M E E R F
NACH6	H C L D I L F A F T K A V L G D S G E L D I L R Q Q M E E R F
SKM1	H C L D I L F A L T K E V L G D S G E M D A L K Q T M E E K F
PN3	H C L D I L F A F T K N V L G E S G E L D S L K T N M E E K F
CARDIAC	H C M D I L F A F T K R V L G E S G E M D A L K I Q M E E K F
SNS2A	H C M D V L F A F T T R V L G D S S G L D T M K T M M E E K F
GLIAL	H C L D I L L A F T K R V M G K D E R V E K I L S E I E S G F

	1990 · 2000 · 2010
RBI	M A S N P S K V S Y Q P I T T T L K R K Q E E V S A V I I Q R
RBII	M A S N P S K V S Y E P I T T T L K R K Q E E V S A I V I Q R
RBIII	M A S N P S K V S Y E P I T T T L K R K Q E E V S A A I I Q R
PN1	M S A N P S K V S Y E P I T T T L K R K Q E E V S A T I I Q R
NACH6	V A S N P S K V S Y E A Y H T T L R R N E E E V S A V V L Q R
SKM1	M A A N P S K V S Y E P I T T T L K R K Q E E V C A I K I Q R
PN3	M A T N L S K A S Y E P I A T T L R W K Q E D L S A T V I Q K
CARDIAC	M A A N P S K I S Y E P I T T T L R R K H E E V S A T V I Q R
SNS2A	M E A N P F K K L Y E P I V T T T K R K E E E Q G A A V I Q R
GLIAL	M L A N P F K I T Y E P I T T T L K R K Q E A V S A T I I Q R

	2020 · 2030 · 2040
RBI	A Y R R H L L K R T V K Q A S F T Y N K N K L K G - - - G A N
RBII	A Y R R Y L L K Q K V K K V S S I Y K K D K G K E - - - D E G
RBIII	N Y R C Y L L K Q R L K N I S S K Y D K E T I K G - - - R I D
PN1	A Y R R Y R L R Q H V K N I S S I Y I K D G D R D - - - D - D
NACH6	A Y R G H L A R R G F I C R K M A S N K L E - - - - - - - - -
SKM1	A Y R R H L L Q R S V K Q A S Y M Y R H S Q D G N - - - D D G
PN3	A Y R S Y M L H R S L T L S N T L H V P R A E E D G V S - - -
CARDIAC	A F R R H L L Q R S V K H A S F L F R Q Q A G G S G L S D E D
SNS2A	A Y R K H M E K M V K L R L K D R S S S S H Q V F C N G D L S
GLIAL	A Y K S Y R L R Q S D K K I Q D I P E I D D G R E D P N S K G

```
               2050                2060                2070
RBI       L L V K E D M I I D R I N E N S I T E K T - D L - - T M S T A
RBII      T P I K E D I I T D K L N E N S T P E K T - D V - - T P S T T
RBIII     L P I K G D M V I D K L N G N S T P E K T - D G - - S S S T T
PN1       L P N K E D T V F D N V N E N S S P E K T - D V - - T A S T I
NACH6     - - - - - - - - - - - - N G G T H R D K K - E S - - T P S T A
SKM1      A P E K E G L L A N T M N K M Y G H E K E G D G - - V Q S Q G
PN3       - L P G E G Y V T F M A N S G L - - - - - P D K S E T A S A T
CARDIAC   A P E R E G L I A Y M M N G N F S R R S A P L S S S S I S S T
SNS2A     S L D V A K V K V H N D - - - - - - - - - - - - - - - - - - -
GLIAL     V H S G Q I E E K A S I Q T Q I - - - - - - - - - - - - - - -

             2080                2090                2100
RBI       A C P P S Y D R V T K P I V E K H E Q E G K D E K A K G K - -
RBII      S - P P S Y D S V T K P E K E K F E K D K S E K E D K G K D I
RBIII     S - P P S Y D S V T K P D K E K F E K D K P E K E I K G K E V
PN1       S - P P S Y D S V T K P D Q E K Y E T D K T E K E D K E K D E
NACH6     S L - P S Y D S V T K P D K E K Q Q R A E E G R R E R A K R Q
SKM1      E E E K A S T E D A G P T V E P E P T S S S D T A L T P S P P
PN3       S F P P S Y D S V T R G L S D R A N I N P S S S M Q N E D E V
CARDIAC   S F P P S Y D S V T R A T S D N L P V R A S D Y S R S E D L A
SNS2A     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
GLIAL     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

            2110                2120                2130
RBI       - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
RBII      R E S K K - - - - - - - - - - - - - - - - - - - - - - - - - -
RBIII     R E N Q K - - - - - - - - - - - - - - - - - - - - - - - - - -
PN1       S R K - - - - - - - - - - - - - - - - - - - - - - - - - - - -
NACH6     K E V R E S K C R R G K E A Y P G T L A S E S L F T N F R I S
SKM1      P L P P S S S P P Q G Q T V R P G V K E S L V - - - - - - - -
PN3       A A K E G N S P G P Q - - - - - - - - - - - - - - - - - - - -
CARDIAC   D F P P S P D R D R E S I V - - - - - - - - - - - - - - - - -
SNS2A     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
GLIAL     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

          2140                2150
RBI       - - - - - - - - - - - - - - - - - - - -
RBII      - - - - - - - - - - - - - - - - - - - -
RBIII     - - - - - - - - - - - - - - - - - - - -
PN1       - - - - - - - - - - - - - - - - - - - -
NACH6     R M Q T A V Q T L A V L E D L Y Q T   P
SKM1      - - - - - - - - - - - - - - - - - - - -
PN3       - - - - - - - - - - - - - - - - - - - -
CARDIAC   - - - - - - - - - - - - - - - - - - - -
SNS2A     - - - - - - - - - - - - - - - - - - - -
GLIAL     - - - - - - - - - - - - - - - - - - - -
```

Figure 6

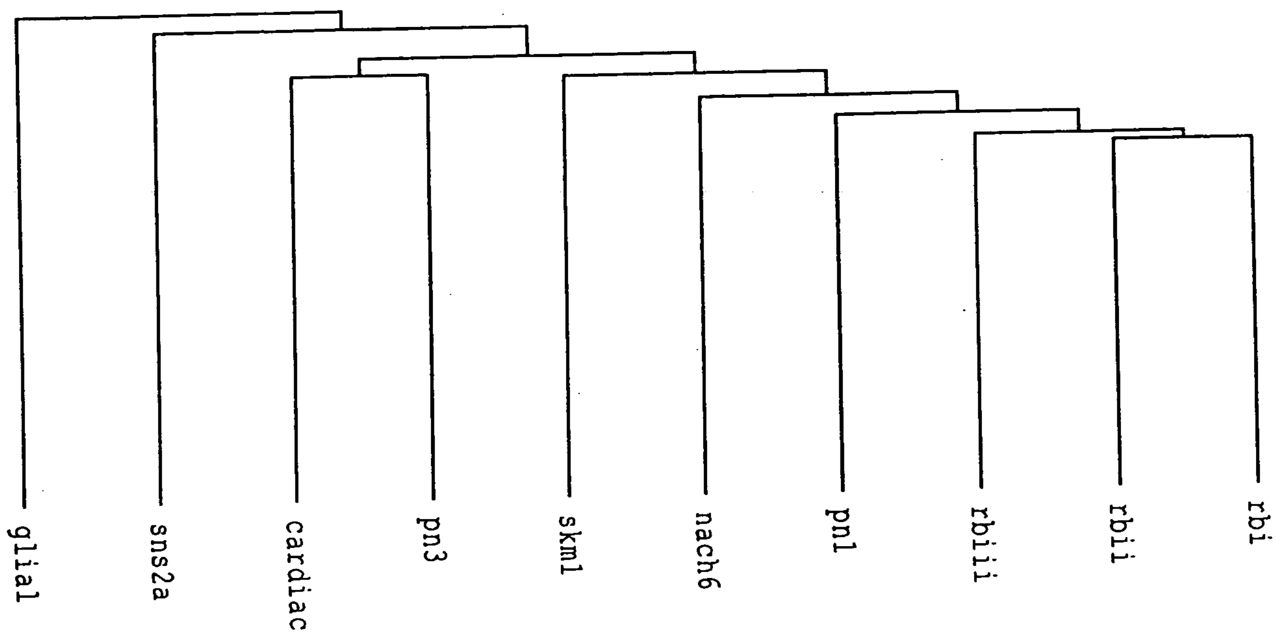
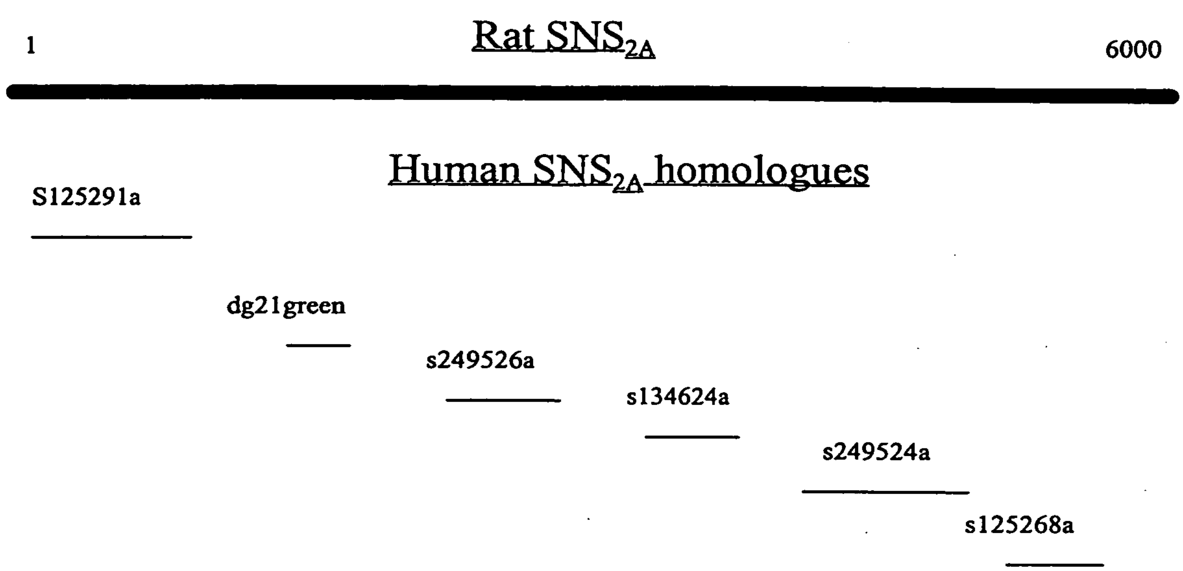


Figure 7



b) Rat SNS_{2A} v s125291a

```
1121. .. ...............GGCTTTACCGACAGATCCTGCGGACCTCTGG 1151
                         || |  |    |||| ||||||·||  ||||
 300 TTTGATATTCAAGAACATGCTCTGTCCTTTCAGACCCTGCGTACTACTGG 251
          .         .         .         .         .
1152 GATCTACTTTGTCTTCTTCTTCGTGGTGGTCATCTTCCTGGGCTCCTTCT 1201
     | ||||||  |||||||||||| | |||||||| |||||||||||||||
 250 GCTCTACTCAGTCTTCTTCTTCATTGTGGTCATTTTCCTGGGCTCCTTCT 201
          .         .         .         .         .
1202 ACCTGCTTAACCTAACCCTGGCTGTTGTCACCATGGCTTATGAAGAACAG 1251
     ||||| ||||| ||||||||||||||||| |||||||| ||||| || |||
 200 ACCTGATTAACTTAACCCTGGCTGTTGTTACCATGGCATATGAGGAGCAG 151
          .         .         .         .         .
1252 AACAGAAATGTAGCTGCTGAGACAGAGGCCAAGGAGAAAATGTTTCAGGA 1301
     ||||  ||||||||||: |||| |||||||||||| || |||||||||||
 150 AACAAGAATGTAGCTGNAGAGATAGAGGCCAAGGAAAAGATGTTTCAGGA 101
          .         .         .         .         .
1302 AGCCCAGCAGCTGTTAAGGGAGGAGAAGGAGGCTCTGGTTGCCATGGGAA 1351
     |||:||||||||||||| |||||| |||||       ||| |  |   |||
 100 AGCNCAGCAGCTGTTAAAGGAGGAAAAAGGA......GGTAGGAAGCCGAA 57
          .         .         .         .         .
1352 TTGACAGAAGTTCCCTTAATTCCCTTCAAGCTTCATCCTTTTCCCCGAAG 1401
     || |:::
  56 TTAANNN...........................................
```

c) Rat SNS_{2a} v dgrc21green

```
1712 GCCCTCAGTGGCTGTGCATAAAGAAGGTCCTGCGGACCATCATGACGGAT 1761
      ||| ||| |||:|:||  |:||:::|||||| | || :| ||||: ||:
 251 .CCCCCAGAGGCNGNGC.GANAGNNNGTCCTGTGTACTNTTATGANTGAN 298
              .         .         .         .         .
1762 CCCTTTACTGAGCTGG.CCATCACCATCTGCATCATCATCAATACCGTTT 1810
     ||  |:| |||||::: ||:|||:|||| ||||::||:|||| :| ||
 299 CCGGTNATTGAGCNNNCCCNTCANCATCAGCATNNTCNTCAACNCTGTCG 348
              .         .         .         .         .
1811 TCTTAGCCGTGGAGCACCACAACATGGATGACAACTTAAAGACCATACTG 1860
     :| | |||:|||:|||:||||| :|||  |:| | ::  :|: :: :: |
 349 NCATGGCCNTGGNGCANCACAAGNTGGGAGNCCAGNNTTNGNGNNGNNGG 398
              .         .
1861 AAAATAGGAAACTGGGTTTTC 1881
     |  :|| :      :
 399 AGTNTAANGGGAACNTG.... 415
```

d) Rat SNS2A v s249526a

```
2052 .........................................AGTGCTG 2058

 325 TTCGCTTAACTGGCTTTTCTCCNTTTTCGTTCGTCGCTTTTTCTACAGCT 276
              .         .         .         .         .
2059 AGGGTCTTCAAGTTAGCCAAATCCTGGCCCACGTTAAACACTCTCATTAA 2108
      |||||||||||    :|||||||||||| || || ||||| || |||||
 275 CAGGTCTTCAAGT..ANCAAATCCTGGCCAACTTTGAACACACTAATTAA 228
              .         .         .         .         .
2109 GATCATCGGCCA..CTCCGTGGGCGCGCTTGGAAACCTGACTGTGGTCCT 2156
     ||| ||| | ||       :|| || || ||||||| ||||||||||||||
 227 GATAATCCGGCAACTCTNGTCGGAGCCCTTGGAAGCCTGACTGTGGTCCT 178
              .         .         .         .         .
2157 GACTATCGTGGTCTTCATCTTTTCTGTGGTGGGCATGCGGCTCTTCGGCA 2206
     |    || ||| |||| || || || || || ||||||| ||| || |||
 177 GGTCATTGTGATCTTTATTTTCTCAGTAGTTGGCATGCAGCTTTTTGGCC 128
              .         .         .         .         .
2207 CCAAGTTTAA..CAAGACCGCCTACGCCACCCAGGAGCGGCCCAGG.... 2250
       | || ||  |    |  |  | |    || | | | |     ||||
 127 GTAGCTTCAATTCCCAAAAGAGTCCAAAACTCTGTAACCCGACAGGCCCG 78
              .         .         .         .         .
2251 ...............CGGCGCTGGCACATGGATAATTTCTACCACTCCTT 2285
                    |||| |||||||||||    |||||| ||||||||
  77 ACAGTCTCATGTTTACGGCACTGGCACATGGGGGATTTCTGGCACTCCTT 28
              .         .         .         .
2286 CCTGGTGGTGTTCCGCATCCTCTGTGGGGAATGGATCGAG 2325
     ||| ||||| |  ||||||||||     |
  27 CCTAGTGGTATCGCGCATCCTCTTGCG............. 1
```

e) Rat SNS2a v s134624a

```
2401 ...................................GGGAAGCTTGTGGTG 2415
                                            |      ||||
  51 TCCTTTGCTAAACTTTCCTTTCTTCTTGCTACCCACCCCATTCCCAGGTG 100
              .         .         .         .         .
2416 CTTAACCTCTTCATTGCCTTGCTGCTCAATTCCTTCAGCAATGAGGAGAA 2465
     || |||||||| |||||||| |||||||||||||| |||||||||| |:
 101 CTCAACCTCTTTATTGCCTTACTGCTCAATTCCTTTAGCAATGAGGTGNG 150
              .         .         .         .         .
2466 GGATGGGAGCCTGGAAGGAGAGACCAGGAAAACCAAAGTGCAGCTAGCCC 2515
        ||| | ||| ||||||||| |||||||||| ||||| ||| |||| |
 151 AACTGGAAACCTAGAAGGAGAGGCCAGGAAAACTAAAGTCCAGTTAGCAC 200
              .         .         .         .         .
2516 TGGATCGGTTCCGCCGGGCCTTCTCCTTCATGCTGCACGCTCTTCAGAGT 2565
     ||||||| ||||||||||| || |  ||  ||   ||| ||||| ||  |
 201 TGGATCGATTCCGCCGGGCTTTTGTTTTGTGAGACACACTCTTGAGCAT 250
              .         .         .         .         .
2566 TTTTGTTGCAAGAAATGCAGGAGGAAAAACTCGCC............... 2600
     || |||  ||||   ||||||| | ||||||  ||
 251 TTCTGTCACAAGTGGTGCAGGAAGCAAAACTTACCACAGCAAAAAGAGGT 300
```

f) Rat SNS2A v s249524a

```
3390 ..........................................TCTCATGA 3397
       |                                         || |
 349 RH.VNTNGAATTNCGAATCTAACCGTCGTACGAGAATCCTGGAATCCTCT 301
              .         .         .         .         .
3398 ATCTACCAAGCTTGAAGTCCTTCCGGACTCTGCGGGCCCTGAGACCTCTG 3447
     | ||     |  | :   :|||||||| :|| || || ||||| |||||
 300 AACTTAATGGAATTNGAANCTTCCGGA.NCTACGAGCACTGAGGCCTCT. 253
              .         .         .         .         .
3448 CGGGCGCTGTCCCAGTTTGAAGGAATGAAGGTTGTCGTCTACG....... 3490
     || |||||||||||||||||||||||||||||          |
 252 CGTGCGCTGTCCCAGTTTGAAGGAATGAAGGTACATTCTGCAGAAGAATG 203
```

g) Rat SNS2a v s125268a

```
3719 .......GGAATGCCTATCTCGCCCTGCTGCAAGTGGCAACCTATAAGGG 3761
              | |     | |    ||  |      ||||   | |||||:
   1 ATCAGTATTATTCATGTTTTTCTGCTTTTTTTGCAGGCACAATTTAAGGN 50
              .         .         .         .         .
3762 CTGGCTGGAAATCATGAATGCTGCTGTCGATTCCAGAGAGAAAGACGAGC 3811
     |||| |||| |:| |  |||| ||||| ||||||| ||||       ||
  51 CTGGATGGATANCGTTTATGCAGCTGTTGATTCCACAGAGGTGAGTCAGT 100
              .         .         .         .         .
3812 AGCCGGAC.......................................... 3819
       :|   |
 101 GTNCTACCATGTTCNNNAGTGTTATGGTCAAGTCAGAGATATCATGACTA 150
```

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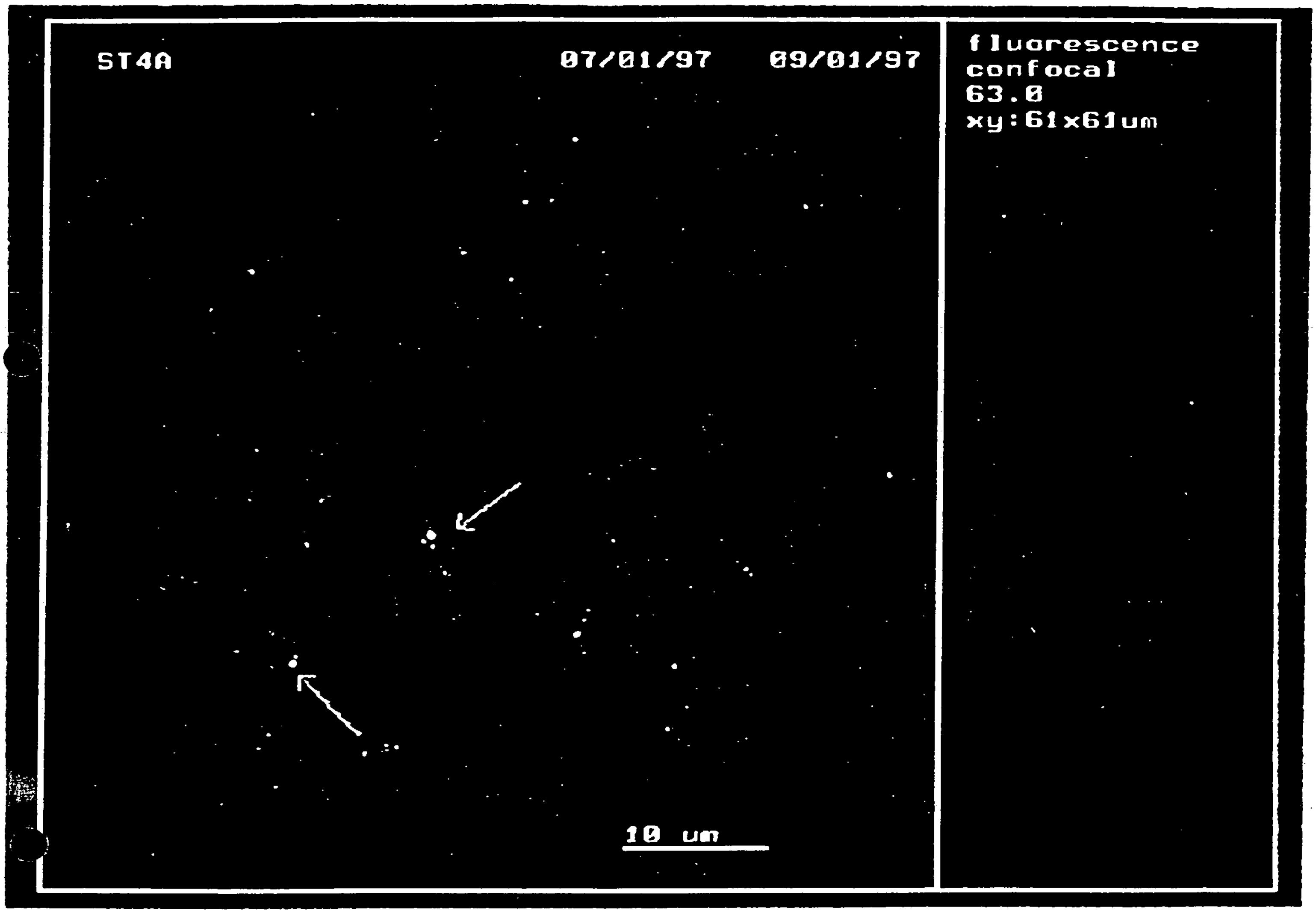
Figure 8

1/1


ST4A
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09/01/97
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confocal
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xy:61x61um
10 um

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Figure 9

1/1

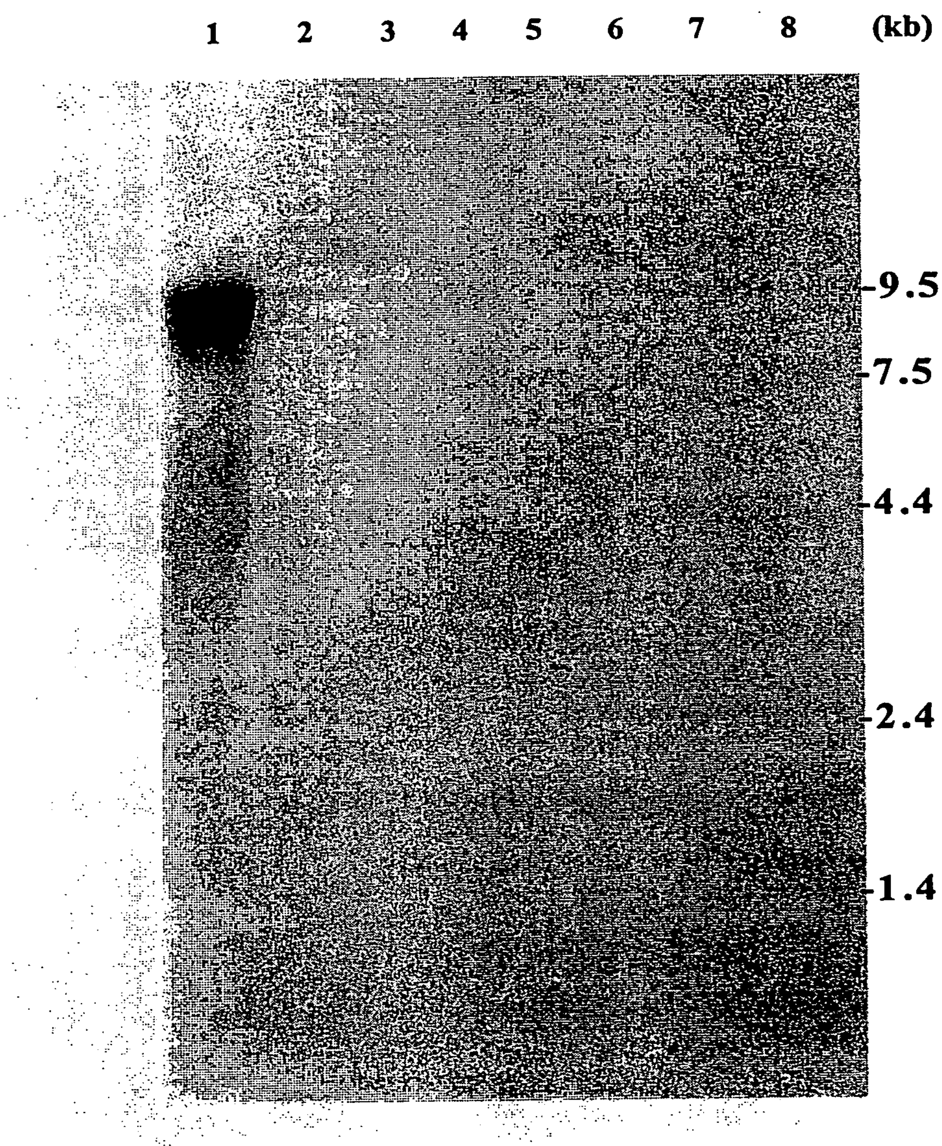
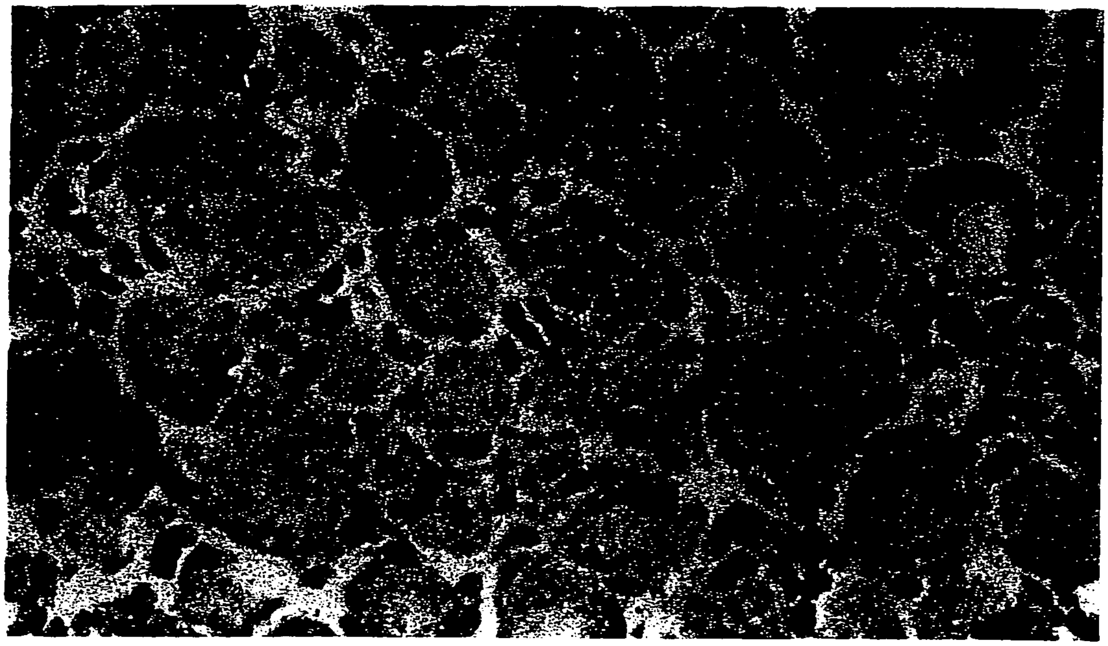


Figure 10

a



b

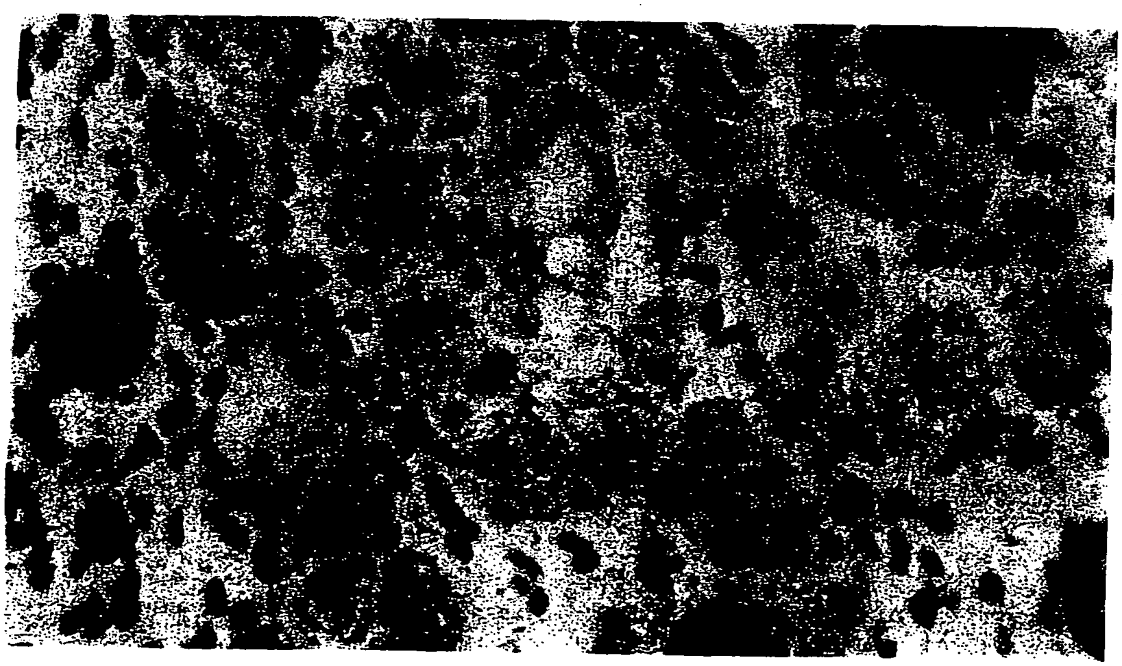


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Figure 11

1/1

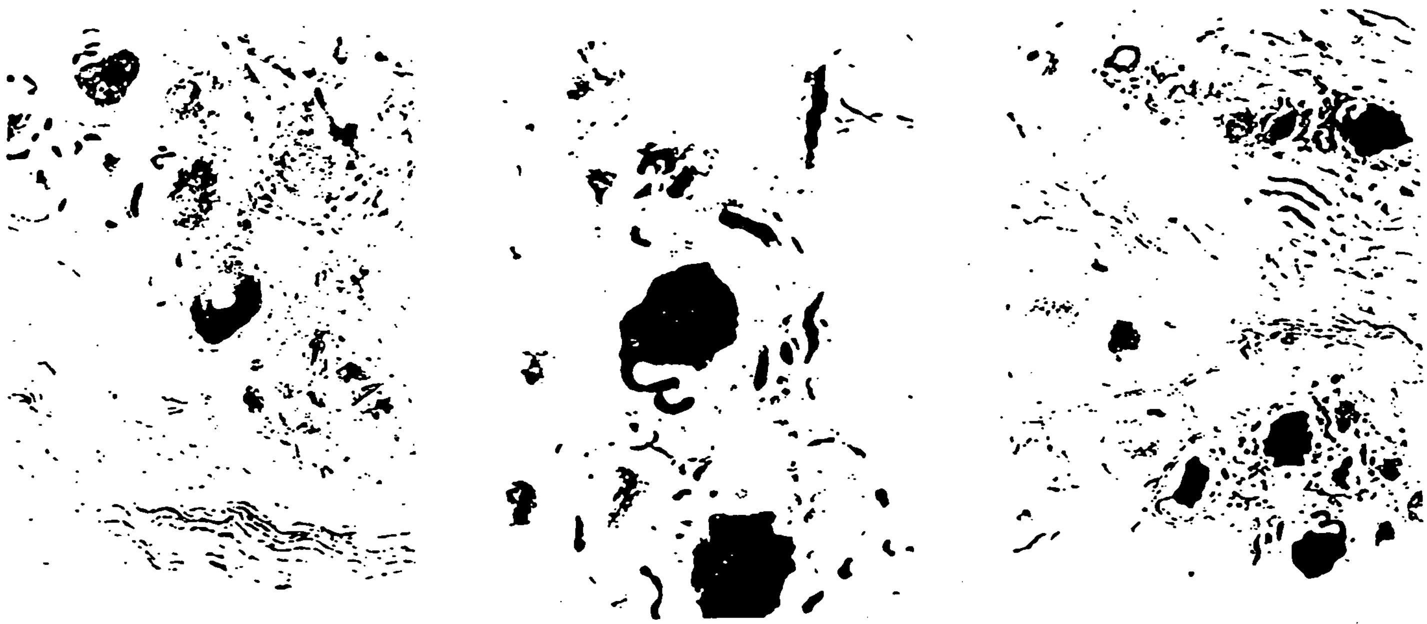
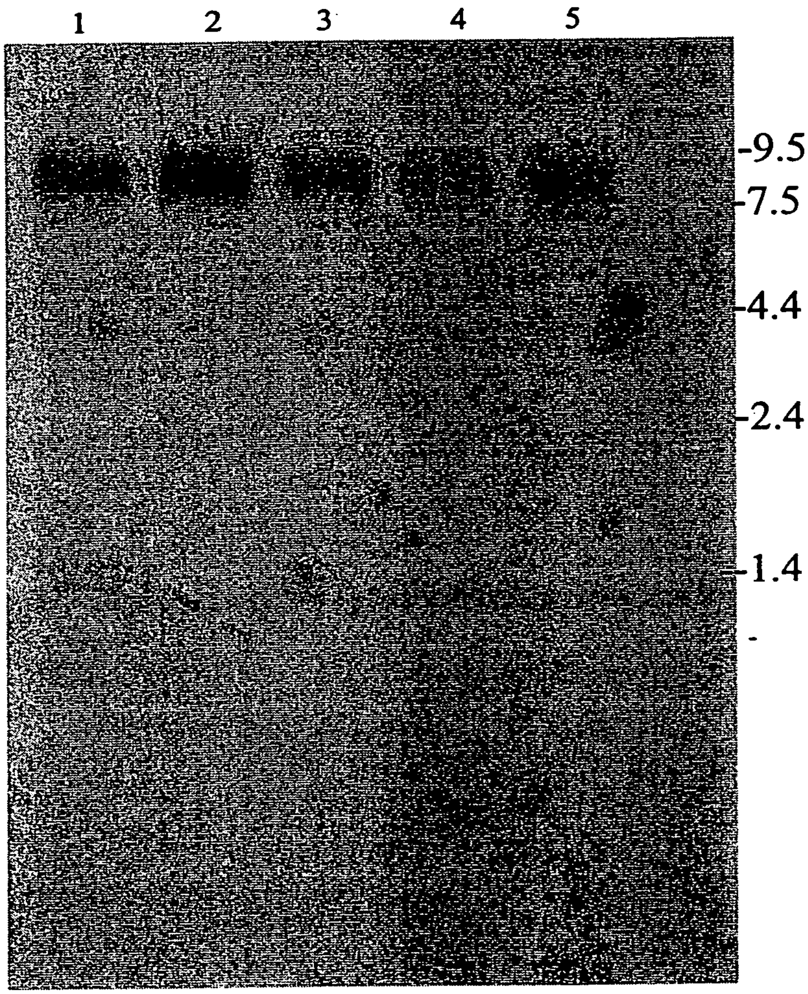


Figure 12

1/1



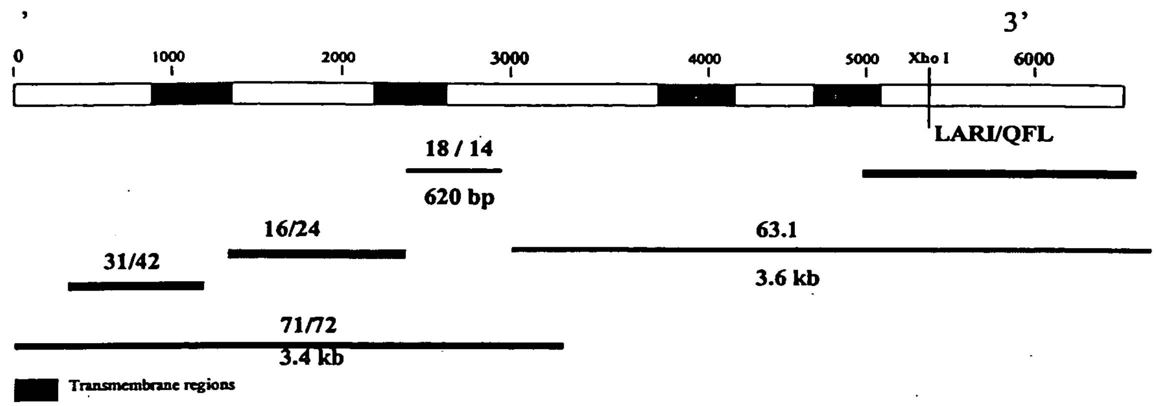
Lane 1 Control DRG
Lane 2 DRG + 24 hours complete freunds adjuvant (CFA)
Lane 3 DRG + 24 hours sciatic nerve cut
Lane 4 DRG + 48 hours sciatic nerve cut
Lane 5 DRG + 7 days hours sciatic nerve cut

PCT/GB99/00839

Glaxo Wellcome plc

8/3/99

Figure 1


3'
0
1000
2000
3000
4000
5000
Xho I
6000
LARI/QFL
18 / 14
620 bp
16/24
63.1
31/42
3.6 kb
71/72
3.4 kb
Transmembrane regions